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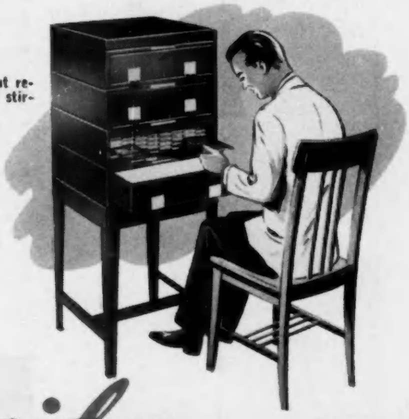

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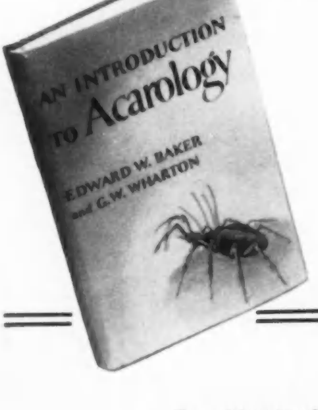

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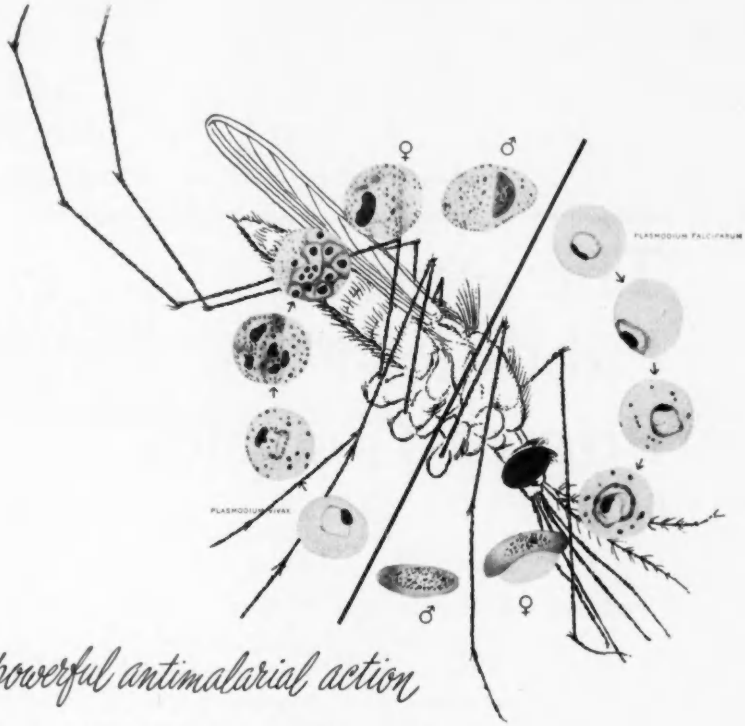

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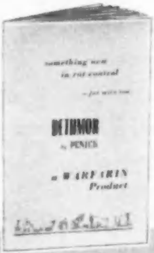

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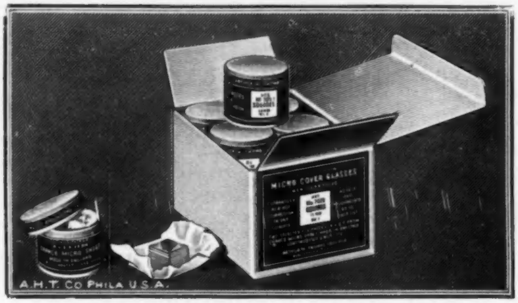

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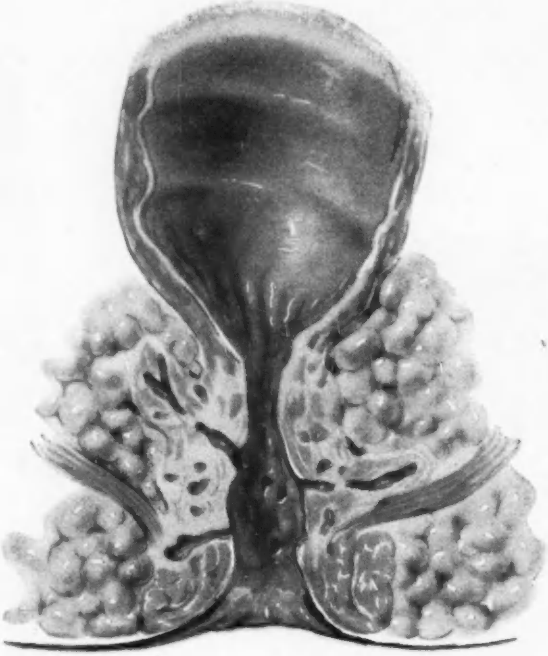

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ERRATA

In the article by Paul G. Weinstein and John G. Appelget, "Some Observations on *Diphyllobothrium Latum* from Shagwa Lake, Minnesota," Volume 1, No. 2, March, 1952, two errors occurred.

1. The address of John G. Appelget, on page 302 should read Grand Rapids, *Minnesota*, not Michigan.

2. On page 305, 9th line from the bottom, the word "proven" should read *unproven*.

THE ELEPHANT NEVER FORGETS[1]

FRED L. SOPER

Hans Zinsser, in 1935, when louse-borne typhus was of no concern to health workers in the United States and DDT was still unknown as an insecticide, wrote:

> "Typhus is not dead. It will live for centuries, and it will continue to break into the open, whenever human stupidity and brutality give it a chance as, most likely, they occasionally will. But its freedom of action is being restricted and, more and more, it will be confined, like other savage creatures, in the zoological garden of controlled diseases."

I have taken the long-memoried elephant as the symbol of the savage communicable diseases, whose activities are being more and more restricted but which have never lost their instinct and capacity for human destruction.

Within five years of Zinsser's prophecy, World War II, with its excess of "human stupidity and brutality," unleashed typhus in Poland, Russia, Iran, Egypt, North Africa, Italy; in the concentration camps of Germany and Austria, and in Japan. Over 300,000 cases occurred between 1942 and 1946 in countries occupied by United States troops, and only the timely development of the chemical attack on the louse avoided a widespread major catastrophe, with millions of cases such as had occurred after World War I (Bayne-Jones 1947).

While louse-borne typhus may possibly continue for centuries, the chemical attack on the louse, spear-headed by DDT, is so popular with infested populations, that there would seem to be a possibility of eventual eradication, in spite of delayed relapses of the type of Brill's disease, unless the recent disturbing report from Korea, of body lice resistant to DDT, be confirmed. Should a resistant strain of lice be spread to other regions of the world by soldiers of the United Nations returning from Korea, it might well be one of the most disastrous results of the Korean War.

Although typhus did not become a problem in the United States during World War II, this country would not necessarily escape, should the United States itself come under attack in some future war. The destruction of housing, followed by inevitable overcrowding, with shortages of water, fuel and especially of soap and insecticides, might well cause a return of epidemic typhus in the United States.

Zinsser, in 1935, did not urge that the zoological garden of controlled diseases be cleared through eradication of its savage creatures, as he surely would do were he alive today, with full knowledge of residual insecticides, antibiotics, chemotherapeutic substances and proven examples of eradication. How Zinsser, who was at heart a public health worker, would have been thrilled by last year's Symposium in Savannah on "Nation-Wide Malaria Eradication Projects in the

---

[1] Presidential address, American Academy of Tropical Medicine, Chicago, Illinois, November 16, 1951.

Americas"; and by the recent liquidation of malaria as a Public Health problem in the United States, which is dramatically signalized here in Chicago this week by the amalgamation of the National Malaria Society and the American Society of Tropical Medicine to form the new American Society of Tropical Medicine and Hygiene.

As responsible public health workers, we must not be misled by the inactive status of certain diseases, no matter how long such apparent inactivity may persist, so long as the conditions suitable for their recurrence are permitted to continue.

Before visiting the Bwamba forest of Uganda some years ago, I was told the story of an English hunter in Africa who, having paid his £50 fee, was out for elephant. The first tusker sighted was a beautiful specimen but was inactive—was, in fact, sitting down. Not only was this elephant sitting, but he failed to rise and become a proper target even after a gentle cough or two from the hunter. Of course, a sitting elephant could not be shot any more than a sitting bird, so the hunt for an active target went on. But the second tusker was also sitting down and likewise showed no inclination to rise. When, at last in desperation, the hunter poked him with the muzzle of his rifle, the elephant is reported to have said: "Oh, go away and leave us alone. Can't you see we're playing book-ends?"

With this pacific tale fresh in mind, I was rudely brought back to reality late one night in the forests of Uganda by the sudden braking of the station-wagon in which I was riding together with a number of mosquito-catchers when, unexpectedly, the rear of an elephant as large, in the headlights, as a barn, loomed suddenly out of the darkness as the highway made a sudden bend. The car was stopped, the lights doused and the party remained in complete silence for 4 or 5 minutes until the great beast had had time to amble off into the forest. Only then did the driver, who had never heard of elephants playing bookends, have the courage to proceed.

Each of the communicable diseases, which have come to mean so little to many of the health workers in the USA and certain other favored countries, is still a dangerous enemy of many millions in other lands.

Yellow fever, like typhus, is not dead in spite of all of the conquests and conquerors of this disease, nor is it likely to die out so long as the tropical and subtropical forests maintain their mammalian hosts and insect vectors. Jungle yellow fever remains as a potential source of virus for undoing the conquest of urban yellow fever, wherever *aegypti* is permitted to continue, as witness the 1928–29 outbreak in Rio de Janeiro, the scene of Oswaldo Cruz's signal triumph two decades earlier. All too many health authorities have thought of yellow fever as "playing bookends" so long as it occurs only as jungle yellow fever. It is in every way as serious to the laborer, infected in the forest, as *aegypti*-transmitted yellow fever is to the person infected in the city, and urban outbreaks, arising from the chance introduction of the jungle virus, are classical in symptoms and in severity.

Since November 1948, a small series of cases of jungle yellow fever have been diagnosed in the Republic of Panama (Courtney 1950). The first cases found occurred east of the Canal Zone, with each succeeding case further west, the last occurring close to Almirante, near the frontier with Costa Rica, in June 1951.

A study of the distribution of immunity to yellow fever in monkeys, begun shortly after the first cases were reported and working ahead of the apparent movement of the disease from east to west, indicated that animals in the forests of all parts of Panama had been previously infected (Clark 1950). At Almirante, immune monkeys were shot over a year before a human case was found there.

With the monkey immunity survey results positive for almost all of Panamá, plans were made for a similar survey of Mexico and Central America beginning in Mexico and continuing south and east, country by country, until immune animals were found. It would then be obvious that the forests intervening between this point and Panamá were also subject to yellow fever. The first study made at Palenque and Cintalapa, in Chiapas, in March and April of this year, showed up immune animals at both points (Clark 1952). Before these results were available, cases of yellow fever began to be observed in Costa Rica in June, not far from the Panamanian frontier and on the Caribbean side of the Continental Divide.

The activity of yellow fever has been much more apparent in Costa Rica than in Panamá and over 150 cases are known to have occurred during the past five months, with each new group of cases found progressively further west and north, until quite recently, when cases have appeared south of the Divide on the Pacific slope. The Costa Rican outbreak has been accompanied by reports of an epizootic among monkeys and the finding of the lesions of yellow fever in the livers of animals found naturally infected in the forests (O. Vargas and Colonel Elton 1951).

The observations in Costa Rica give strong support to the suggestion, based on the scattered Panamanian cases, that yellow fever is working from east to west and northwest, in an epizootic wave, remaining in a given area only a matter of weeks or, at most, months, and burning itself out, apparently through exhaustion of the supply of susceptible animals.

Such an epizootic wave was observed from 1934 to 1940 in the Brazilian States of Mato Grosso, Goiaz, São Paulo, Minas Gerais, Parana, Santa Catarina, Rio Grande do Sul, Rio de Janeiro and Espirito Santo, in Paraguay, and in the Province of Misiones, Argentina.

On the other hand, certain areas may, apparently because of large numbers of short-lived highly reproductive susceptible mammals, maintain jungle yellow fever as a permanent enzootic, as in Ilhéos, Brazil, and in Muzo and San Vicente de Chucurí, Colombia. In Ilhéos, there is reason to believe that, in the small rain-forest cacao-producing zone comprising only 2 or 3 counties, the virus of yellow fever has been constantly present during the last 20 years. In the same way the county of Muzo, Colombia, which has had yellow fever reported intermittently since 1909, reacted to viscerotomy in the three years before systematic vaccination was introduced with human cases every year and, in one or another of the three years, in every month of the calendar. San Vicente de Chucurí, which first came under scrutiny with yellow fever deaths in 1933, has had fatal cases in ten of the past 18 years and, in 1951, had deaths in January, May, July, August and October (PASB Epid. report 1951).

It remains to be seen whether the present Panamá-Costa Rica epizootic will be traced by the observation of human cases through Nicaragua, Honduras and Guatemala into Mexico, or whether its movement may be blocked by some intervening zone of animal immunity, due to a previous local enzootic.

The observance of jungle yellow fever in Panamá and Costa Rica, and the finding of immune monkeys in Mexico, has not altered the basic picture of yellow fever in the Americas; rather do they complete the jigsaw mosaic pattern of jungle yellow fever in the Americas almost 20 years after the first isolation of the yellow fever virus, in the absence of *Aëdes aegypti*, in the Valle do Chanaán, Brazil, in 1932 (Soper, 1937).

Looking at the 20 year map of the distribution of known yellow fever in the Americas, and noting how recently Central America and Mexico have fitted into the picture; how, in 1949, an outbreak of over 800 cases appeared in southern Bolivia in a valley where yellow fever had not occurred during the past 18 years and how, in 1951, the first outbreak of jungle yellow fever ever to be registered west of the Andes occurred in Ecuador (Garcia 1951), in the coastal region where no yellow fever at all had been found since 1919, it would appear that the yellow fever elephant never permanently forgets.

Today we know that jungle yellow fever, an important public health problem in its own right and a permanent potential source of virus for the reinfection of towns harboring *aegypti*, occurs from time to time in all of the suitably inhabited tropical and subtropical forested areas of Mexico, Central America, Panamá and South America. In South America, only Chile and Uruguay are free from this disease. Jungle yellow fever has been known in Colombia and Venezuela for many years, within easy striking range of the United States and of the West Indies by air transportation; and the jungle infection in Panamá, Central America and Mexico does not significantly increase the threat, so long as the cities and towns of the infected countries remain free from infection. Fortunately, Panamá City was practically free of *aegypti* when the infective cases came to the Santo Tomás Hospital in 1948, and the 1951 outbreaks in Ecuador and Costa Rica found the campaigns for the eradication of *Aëdes aegypti* already under way, not only in these countries, but also in the other countries of Central America and in Mexico.

Following the discovery of yellow fever at Almirante, close to the Costa Rican frontier, the health authorities of Costa Rica began an intensive vaccination campaign among the rural residents in areas lying in the anticipated route of invasion. In the more isolated districts, where refrigeration for the preservation of vaccine often was not available, the Dakar neurotropic vaccine was used in the belief that it is more stable than the 17D vaccine.

A number of typical cases of yellow fever have been observed in vaccinated persons beginning as long as four and five weeks after vaccination. This is in contrast to the field experience with the 17D vaccine which, used in epidemic areas, has apparently prevented infections after only a few days, leading to the conclusion that the vaccinated individual is fully protected by the end of a week (Soper *et al.*, 1938). Likewise, recent studies with 17D virus (Hahn 1951) show that this vaccine prevents the circulation of the Dakar virus if a period of only four days intervenes between the applications of the two viruses. It seems logical to conclude that the failure with the Dakar vaccine indicates that it had not survived exposure to field conditions, or had been applied ineptly.

A series of cases of encephalitis occurring in persons vaccinated with the Dakar vaccine in Costa Rica and, more recently, in Honduras, is now being studied to determine the nature of this encephalitis. Until further data become available, vaccination in Central America is being limited to the use of the 17D chick embryo vaccine by injection.

There is real need for greater comprehension of the tragedy of jungle yellow fever and of the necessity for the routine vaccination of the relatively inaccessible populations who live and work in contact with the tropical forests of the Americas.

A tragic occurrence early this year was an outbreak of jungle yellow fever with over 2000 cases and several hundreds of deaths, among the newly arrived settlers in the State of Goiaz, Brazil. This area had suffered an outbreak in 1945, at which time extensive vaccination was carried out in the then relatively small population. During the past six years, however, an important agricultural development has brought in large numbers of nonimmunes from Northeast Brazil. Traveling overland, these newcomers passed through no place where routine vaccination would reach them. Ironically enough, these newcomers came from the previously highly endemic region of *aegypti*-transmitted yellow fever of Northeast Brazil, where there is no jungle yellow fever. It was here that the Rockefeller Foundation initiated its campaign in Brazil against *aegypti*-transmitted yellow fever in 1923, with the resultant development of this nonimmune susceptible population.

Much greater attention would be given to the vaccination of all exposed populations and more ready collaboration in the campaign for the eradication of *Aëdes aegypti* would occur, if jungle yellow fever more regularly caused outbreaks of urban yellow fever. Health authorities have short memories and tend to forget the outbreaks in Rio de Janeiro, 1928–29; in Guasapati and Tumeremo, Venezuela, 1929; in Socorro and Simacota, Colombia, 1929; in Santa Cruz de la Sierra, Bolivia, 1932; in Cambará, Paraná, Brazil, 1936; in Buena Vista, Colombia, 1937; and in Senna Madureira, Brazil, 1942, all of which are attributed to ubanization of the jungle virus.

As the years pass and jungle yellow fever continues to be jungle yellow fever, without invasion of the cities, the potential threat seems unimportant to many who do not realize the tremendous effort which has gone, and is going, into the eradication of *Aëdes aegypti* in all of the towns and cities in close contact with the infected forests. Years ago, the yellow fever workers in Brazil discovered that the only permanent friend of the Yellow Fever Service was yellow fever itself. The very success of the program for the eradication of *Aëdes aegypti* in some areas in preventing urban yellow fever, militates against the completion of the task in other regions.

My attention has recently been called to an address, by the Dean of the School of Public Health of Harvard University (Simmons 1951) in which the following paragraph from the inaugural address of the President of the American Medical Association, June 1950, is quoted:

> "Dread diseases like typhoid, diphtheria and smallpox which, 50 years ago, took a heavy toll in sickness and death, virtually have been eliminated as national health problems and all the infectious diseases have been brought under effective methods of prevention, control and treatment."

Dean Simmons challenges this as:

> ". . . a statement so obviously incorrect, that it deserves comment only because it comes from an official authorized to speak for the leading medical organization of the country."

He then calls attention to 1) the occurrence of a group of diseases which have not been brought under effective methods of prevention including poliomyelitis, measles, the common cold and infections of the respiratory tract, and 2) the continued occurrence of another group of diseases for which adequate methods of prevention are now available, including intestinal infections, insect-borne infections and the venereal diseases.

In other words, returning once more to Zinsser's figure, the distinction is made between two groups of savage creatures, the first, as yet untamable; the other already "confinable in the zoological garden of controlled diseases."

The statement is then made, referring of course only to the second group, that

> ". . . The American people are not yet deriving the full benefit of our present-day knowledge about the prevention of disease and, that we have a long way to go before we dare announce that the infectious diseases are really under control."

But when we analyze the figures given by Dean Simmons for these controllable diseases in relation to the population at risk, or in relation to the number of practicing physicians, it is found that the control is excellent and that what is being demanded, is really the eradication of these diseases.

The criticism of the statement of the President of the American Medical Association becomes then a criticism of the members of the sister organization, the American Public Health Association, who have not completed the task of preventing the preventable diseases and possibly a criticism of the Harvard School of Public Health which, together with similar faculties, has not yet created a generation of health workers trained to distinguish between eradicable and noneradicable diseases, and to push on to eradication when possible.

That the American Public Health Association is not fully alive to the possibilities of eradication as was the National Malaria Society, is evident from the records of the recent 79th meeting of the APHA in San Francisco when, with registration of over 4000 members and with a multiplicity of sections, only 30 papers were presented on all phases of communicable diseases and no general analysis of future possibilities was made.

Of these papers, 17 related to diseases for which more or less adequate methods of control are available:

Brill's Disease,	1
Brucellosis,	1
Diphtheria,	2
Infantile Diarrhea,	1
Pertussis,	1
Rabies,	2
Salmonella,	1
Shigella,	1
Smallpox,	1
Syphilis,	1
Tuberculosis,	4
Yaws,	1

Lest the picture I paint may seem too somber, it should be pointed out that bovine tuberculosis in humans, which was so common in the hospitals of Chicago when I was an interne, has become a rarity here and this city has announced that, beginning January 1, 1954, all milk sold here must come from brucellosis-free herds. At least there is the conviction here that animal diseases can be eradicated. It should also be mentioned that some workers in tuberculosis and venereal disease are seriously beginning to consider the possibility of what might be termed "area-eradication" of these diseases. But "area-eradication" calls for permanent protection from reinfection or reinfestation from other areas or other countries. The alternative is to undertake eradication as an ever expanding program, as has occurred in the case of the program for the eradication of the *Aëdes aegypti* mosquito during the past 18 years, on a regional and, possibly, eventually on a world basis.

Such expanding eradication requires a mechanism through which the affected countries can unite on common programs, approved and supported by all, with identical technical orientation and with no loss of sovereignty to any nation. Fortunately, there already exists such a mechanism, based on treaties ratified by practically all of the countries of the Americas and of the World, ready to function in the co-ordination of such programs; I refer, of course, to the mechanism of the official international health agencies, the Pan American Sanitary Bureau and the World Health Organization.

REFERENCES

1. Bayne-Jones, Stanhope 1947, The control of typhus fever (U. S. Typhus Commission) *Rhode Is. Med. J.* **30**: 423–436.
2. Clark, Herbert C. 1950, Search for the wild animal reservoir of yellow fever in Panama, Annual rep. of the Gorgas Memorial Lab., p. 3.
3. Clark, Herbert C. 1952, Endemic yellow fever in Panama and neighboring areas *Am. J. Trop. Med. Hyg.* **1**: 78–86.

4. COURTNEY, K. O. 1950, Report on the recent outbreak of jungle yellow fever in Panama, *Am. J. Pub. Health* **40**: 417–427.
5. GARCIA S., EGBERTO 1951, unpublished report.
6. HAHN, RICHARD G. 1951, A combined yellow fever–smallpox vaccine for cutaneous application, *Am. J. Hyg.* **54**: 50–70.
7. Pan American Sanitary Bureau 1951, *Monthly Epidemiological reports 8:* Nos. 3, 7, 8, 9 and 11.
8. SIMMONS, J. S. 1951, Public health as a weapon for national defense, *Trans. Coll. Phys. Phila.* **19**: 1–13.
9. SOPER, F. L. 1937, Yellow fever without *Aedes aegypti;* study of a rural epidemic in Valle do Chanaan, Espirito Santo, Brazil, 1932, *Am. J. Hyg.*
10. SOPER, F. L., AND SMITH, H. H. 1938, Vaccination with virus 17D in the control of jungle yellow fever in Brazil, *Trans. 3d Internat. Cong. Trop. Med. & Mal.* (*Holland*) **1**: 295–313.
11. VARGAS, O., AND COL. NORMAN ELTON 1951, unpublished observations.
12. ZINSSER, HANS 1935, Rats, Lice and History, Little Brown & Co., Boston.

PANEL PAPERS ON RESISTANCE OF INSECTS TO INSECTICIDES

INTRODUCTION

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There could hardly be a more timely subject in the entire field of insect vector control than the present one.

Within the last decade we were within sight of realizing our fondest hopes in the eradication of, or at least effective control of, many arthropod borne diseases and pestiferous insects. The last few years of this decade, however, have dealt a blow to these aspirations by the fact that insects have developed resistance to our most potent chemical weapons. We now must go back and reorganize our forces for a new attack. The following speakers were asked to participate in this Panel not only because of their preeminence in the field, but because their respective interests and work combine to cover almost all phases of the problem confronting us.

First, Dr. A. D. Hess, of the U. S. Public Health Service, discusses the "Significance of Resistance to Insecticides in Vector Control Programs." Dr. Hess is Assistant Chief of the Technical Development Services in the Communicable Disease Center, and prior to his affiliation with the Service he was Chief Biologist, Health and Safety Division, Tennessee Valley Authority. His work and contacts in the field of vector control, his association with operational as well as research programs, and his acquaintance with foreign activities in the field amply qualify him to speak authoritatively on the broad significance of this most important problem.

Two particular groups of insects with which we are most immediately concerned are mosquitoes and flies. Dr. E. F. Knipling, Chief of the Division of Insects Affecting Man and Animals, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, is a recognized authority in the field of vector control. It was under his jurisdiction that the first DDT was used in this country when he was in charge of the Orlando, Florida, laboratory. Dr. Knipling won the U. S. Army Typhus Medal and has recently been honored by election to the Presidency of the American Association of Economic Entomologists. Some of the earliest and principal work on mosquito resistance has been accomplished by his staff, and his address on the "Present Status of Mosquito Resistance to Insecticides" presents the latest knowledge available on this subject.

The other large group of insects with which we are concerned is flies. Dr. George C. Decker, Entomologist and Head of the Illinois Natural History Survey, will discuss the problem of "House Fly Resistance to Chemicals." The names of Dr. Decker and Dr. Willis N. Bruce, who has been associated with him in this work, are familiar to everyone interested in the subject of insecticide resistance. Dr. Decker has not only done pioneer research work in the field but has taken the lead in coordinating research on fly resistance among the principal laboratories of the Nation. His work has been of a practical nature, carried out in the homes and on the farms in Illinois, and we know of no one better suited to handle this complex subject than Dr. Decker.

The chaos caused by the failure of insecticides can be blamed, to a large extent, to the short-sightedness of those who failed to support fundamental research work when it appeared that the problem was solved. The meager amount of basic research work on insect physiology and toxicology during the last ten years has been appalling. It is now necessary not only to find out why our new insecticides have failed, but also to go back and find out why they worked in the first place. One individual who early recognized the need for basic studies of this type was Dr. Leigh E. Chadwick, Chief, Entomology Branch, Chemical Corps Medical Laboratories, Army Chemical Center, Maryland. He has pioneered in conducting physiological studies on resistance of insects to insecticides, and is an active leader in this field of research. It is on the fundamental work of Dr. Chadwick and others like him that we must pin our hopes for the future solution of this problem, and it is fortunate indeed that he is able to be with us at this time to discuss "The Current Status of Physiological Studies on Resistance to Insecticides."

THE SIGNIFICANCE OF INSECTICIDE RESISTANCE IN VECTOR CONTROL PROGRAMS[1]

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INTRODUCTION

The occurrence of resistance to insecticides is not new. John B. Smith apparently observed resistance to kerosene in the San Jose scale in 1887 (Babers and Pratt, 1951). Since 1914, when Melander asked his now famous question "Can insects become resistant to sprays", at least a dozen agricultural species have developed varying degrees of resistance to insecticides. The problem of insecticide resistance did not, however, become of general concern to medical entomologists until some time after DDT had come into widespread use.

It is the purpose of the present paper to present a summary review of the impact of insecticide resistance upon vector control and related programs in various parts of the world and its implications for the future. Pertinent information has been obtained from literature, from personal correspondence and discussions, and from the results of recent investigations being conducted by the Communicable Disease Center. Development of insecticide resistance by certain animal parasites, as for example arsenic resistance of the cattle tick, is not covered in this paper. Although the coverage is not complete, the information presented is believed to be representative of the current status of insecticide resistance in the different regions of the world.

The following definitions are given for the terms which will be used in this paper in referring to the various types of resistance phenomena:

Tolerance. The ability of the individual to withstand normally injurious dosages of a toxicant through previous exposures to sublethal dosages (some authorities have used "mithridatism" with a similar meaning).

Resistance. The ability of a population to withstand a toxicant to a greater degree than a normal population, and to transmit this characteristic from one generation to another. Three general types of resistance are defined as follows:

1. *Physiological*. The ability through biochemical processes to withstand a toxicant after it has entered the body.
2. *Morphological*. The ability through morphological structures to prevent a toxicant from entering the body.
3. *Behavioristic*. The ability through protective habits or behavior to avoid injurious contact with a toxicant.

CURRENT STATUS OF INSECTICIDE RESISTANCE

Italy

After initial use by the allied military government in Italy in 1944, extensive applications of DDT residual sprays were initiated in Italy in the spring of 1946 for the control of malaria mosquitoes (Missiroli, 1950 and 1951). The following year the first DDT-resistant house flies and *Culex pipiens autogenicus* were observed (Sacca 1947; Mosna 1947). Chlordan was used the following year to supplement DDT, being applied to the kitchens, stables, pig sties, etc. (Bettini and Barachini 1948). Excellent results with both flies and mosquitoes were obtained in 1948 and 1949 (Mosna 1949 and 1950). However, in August of 1950 chlordan-resistant flies appeared, and by the end of the year all of the house flies and *Culex pipiens* in the area seemed to be resistant to this chemical. The house flies were also found to be resistant to methoxychlor and dieldrin. By the end of the 1951 season house flies in every malaria region throughout Italy were resistant to DDT, methoxychlor, chlordan, dieldrin, and benzene hexachloride, and essentially any other available residual spray.

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[1] From the Communicable Disease Center, Public Health Service, Federal Security Agency, Atlanta, Georgia.

In spite of the high level of resistance of house flies and *Culex pipiens* in Italy, there is at present no indication of resistance in the local species of *Anopheles*.

Sardinia

The first island-wide application of DDT residual sprays for the control of *Anopheles labranchiae* on Sardinia were made under the technical direction of the International Health Division of the Rockefeller Foundation in the winter of 1946-47 with excellent results against flies, mosquitoes, and other domestic insects (Barnard 1949; Logan 1950). The following season poor results with house flies were observed in certain villages, and re-treatment of some of these with DDT in the late summer of 1948 was ineffective, though chlordan gave good results (Aitken, 1951). In the winter of 1948–49 chlordan was used in some areas and DDT in others, but by the summer of 1949 chlordan- and DDT-resistant flies were present everywhere. These resistant flies returned in fantastic numbers in 1950 and tests made in the fall of that year indicated that in addition to DDT and chlordan, they were also resistant to dieldrin, aldrin, benzene hexachloride, and toxaphene (Busvine, 1951).

There is apparently no evidence of the development of any significant degree of resistance by the local anophelines, although Trapido (1951) has shown that *A. claviger* is naturally able to withstand considerably higher dosages of DDT than *A. quadrimaculatus*.

Greece

The first generalized application of DDT in Greece in 1946 gave phenomenally effective control of house flies and other household pests. However, DDT-resistant flies were observed in 1947, and resistance subsequently appeared in the house mosquito, *Culex molestus* (*C. pipiens autogenicus* Roubaud), and in *Pulex irritans* (Hadjinicolaou, 1951). The appearance of DDT-resistant sandflies, *Phlebotomus papatasii*, was reported in 1949 (Brown, 1951A).

Hadjinicolaou is of the opinion that *Anopheles sacharovi*, the most important vector of malaria in Greece, has begun to show resistance to DDT. He states that this species is now found in considerable numbers quietly resting on treated surfaces for the first time since extensive residual spraying was initiated in 1946, and that there is a high percentage of survival of mosquitoes collected from treated surfaces. For the first time, *Anopheles maculipennis* has also been found in small numbers in sprayed shelters. *Anopheles superpictus* and culicines other than *C. molestus* do not as yet show any signs of resistance.

Denmark

In Denmark DDT has been used for fly control on farms since 1944 and has been in extensive general use over the entire country since 1946. Control of house flies with DDT residuals failed in some cases in 1946 (Keiding and van Deurs 1949). Resistance spread rapidly through the summer of 1948, and by the spring of 1949 most of the farms had DDT-resistant flies. The presence of this resistance was first confirmed by laboratory tests in the early part of 1948. BHC-resistant flies appeared in October, 1949, about three months after the initial use of this material, and chlordan-resistant flies appeared the same year. By July of 1950 fly populations were found which had acquired resistance to DDT, BHC, and chlordan, and also showed high resistance to toxaphene and dieldrin (Pal 1951A).

Sweden

Wiesmann (1947) discovered a resistant strain of house flies in Sweden in 1946 in an area north of Stockholm where DDT had previously not been used. In a recent communication Dr. Wiesmann (1952) states that he believes this to be a case of natural rather than acquired resistance.

England

Field strains of house flies collected in 1949 from farms near Stratford-on-Avon and Winchester were reported to be resistant to DDT (Harrison 1950). However, there is little scientific information available on the extent to which flies in England have become resistant to DDT. Under the prevailing climatic conditions the house fly problem is not severe, and there is no extensive routine application of DDT, which may be a factor in limiting the rate at which resistance has developed (Busvine 1951A).

Egypt

A 2.4 per cent gamma BHC dust was applied at Quaranfil, Egypt, at approximately monthly intervals from October, 1948, for the control of larvae and adults of *M. domestica vicina* in an area where previously there had been no general use of DDT or other insecticides. The treatments became ineffective 10 to 12 months later, and laboratory tests confirmed that the flies were highly resistant to BHC (Gahan and Weir 1950). Chlordan was used in 1950. In April, 1951, applications of BHC were initially effective, but became ineffective within a few weeks (Weir 1951). In another group of villages in the Giza Province in 1951 house flies became highly resistant to BHC after five treatments in seven months.

Chlordan was used effectively in villages in the Sindibis area during 1950, but by the third treatment in July of 1951 the flies had become resistant to this insecticide, and BHC was also ineffective. Although residual applications of

DDT had been made in Sindibis prior to the use of BHC, there was no essential difference in the time required for the development of resistance there and in Quaranfil, where DDT had not been used. There appears to be no sound evidence that a DDT-resistant house fly has developed in Egypt (Weir 1951).

West Africa

Several years ago Muirhead Thomson (1947; 1947A) reported on observations with *Anopheles gambiae* and *A. melas* in Nigeria, West Africa, which showed that the number of mosquitoes found resting inside DDT-treated habitations was not necessarily a criterion of the effectiveness of the measure. As soon as four days after treatment, mosquitoes were able to enter the houses, take a blood meal, and escape to the outside without having obtained a lethal exposure. This was apparently due to the "irritant-repellent" effect of the DDT-kerosene formulations used. More recently Hadaway (1950) has shown that engorged mosquitoes, including *A. gambiae*, were able to leave DDT-treated native huts in Uganda without obtaining a lethal dose. This pattern of behavior, whether due to irritant-repellency of DDT or to an inherent tendency, may through natural selection become increasingly effective in protecting the species from indoor residual sprays. Such behavioristic resistance may be as important for some species as the more generally recognized physiological type of resistance.

Levant States

It is reported that DDT and benzene hexachloride are now ineffective against house flies in U. N. refugee camps in the Levant (Brown 1951).

India

It is of particular interest that house flies (*M. nebulo* and *M. vicina*) in India have apparently not become resistant to DDT as indicated by recent correspondence with Colonel Jaswant Singh and Dr. Rajindar Pal of the Malaria Institute (1951). Regular spraying of houses with DDT for malaria control was started at Delhi and other parts of India in 1945 and has been extensively used since. *M. nebulo* collected from areas sprayed for four consecutive years has shown no DDT resistance in comparison with a normal laboratory colony (Pal 1951). Laboratory studies indicate that *M. nebulo* does not develop resistance to the same degree as does *M. domestica*. Another possible factor in the failure of Indian house flies to develop resistance is that DDT residual spraying is limited to the three or four months of the malaria season and the application rate is only 50 mg./sq. ft. (Pal 1951). There is at present no indication that field strains of mosquitoes have developed any resistance to DDT in India.

Ceylon

Routine application of DDT residual sprays to houses for the control of *Anopheles culicifacies* was initiated in large areas of Ceylon about April of 1947. Observations made two years later indicated that *M. vicina*, the dominant house fly in that area, had not developed any resistance to DDT (Busvine 1949).

Korea

The use of DDT to eliminate a typhus epidemic in Naples in 1943 has become a classic in the annals of disease control. It is, therefore, of particular interest to note the recent report that 10 per cent DDT powders are not effective against the human body louse (*Pediculus humanus corporis*) in Korea (Hurlbut, Altman, and Nibley, 1952). Although the application method was essentially that used at Naples, treatment of Korean military personnel in the winter and spring of 1951 resulted only in an increase of infestation. Subsequent tests indicated that the Korean lice were quite resistant to DDT in comparison with the Orlando, Florida, strain as reported by Bushland *et al.* (1945). The high DDT resistance of the Korean body lice was confirmed by Eddy (1951) who found, however, that they were about as susceptible as the Orlando strain to TDE, allethrin, pyrethrins, toxaphene, chlordan, lindane, aldrin, and dieldrin. Head lice (*P. humanus capitis*) collected from Korean and Chinese refugees were susceptible to DDT.

At present it has not been determined whether the resistance of the Korean body lice to DDT is a natural characteristic of the strain or if it has been acquired through previous exposure to DDT.

Reports currently available do not give any indication of the development of DDT-resistant flies or mosquitoes in Korea.

Japan

In Japan, as in other areas, 5 per cent DDT residual sprays are used for fly control around U. S. Army installations. The scheduled applications are usually made at 30 to 60 day intervals. No major difficulties with resistance of flies, roaches, mosquitoes, or other household pests have been reported when these residual sprays were applied in accordance with the recommended schedules (Reed 1952).

Australia

From the few observations which have been made, it would appear that highly resistant strains of house flies have not as yet developed in Australia. There are, however, a few localized indications of the occurrence of house flies with some degree of resistance to DDT, as for example in New South Wales, where a few *M. domestica* collected in May, 1950, were found to be more than 30 times as resistant to topical applications of DDT as a laboratory colony (Waterhouse 1951).

There are no reports of the development of insecticide resistance by mosquitoes in Australia. The apparent scarcity of insecticide-resistant strains may be due to the fact that most control programs are directed purely against pest mosquitoes and are run rather casually (Mackerras 1951).

South America

DDT-resistant house flies have been reported from several countries in South America, and though no general survey has been made, it appears that this species has or will develop resistance wherever there is widespread use of DDT residual sprays.

In Perú, where DDT residual sprays have been applied for malaria control since 1946, flies are now highly resistant, and there are suggestions that *Pulex irritans* is developing resistance (Russell 1949; Villalobos 1951). *Triatoma* seems to possess a natural resistance to DDT and to a lesser extent to chlordan and dieldrin, but BHC at 500 mg./sq. meter is effective for about 3 months. There is no indication of resistance in anopheline mosquitoes or *Phlebotomus verrucarum*, the vector of bartonellosis.

In Argentina the first general application of DDT residual sprays was made in August, 1947, continuing through the season into the early part of 1948. Alvarado (1951) estimates that approximately three-fourths of the house flies in the treated areas are now resistant to DDT. Dr. Alvarado has also observed that in the northern part of Argentina where *Anopheles darlingi* is present, this species is showing an increasing tendency to rest on the outside untreated surfaces rather than on the inside treated surfaces of dwellings. This would constitute a behavioristic type of resistance similar to that noted for *A. gambiae* in Africa. A similar observation on the resting habits of *A. darlingi* was made by Bustamante *et al.* (1951) in Brazil in the state of Minas Gerais where DDT residuals have been applied regularly to the interiors of houses since the 1946–47 season. Experiences of the National Malaria Service of Brazil in their residual spray program indicate the development of resistance in house flies and some culicine mosquitoes, but DDT is still effective in the control of the local anophelines (Pinotti 1951).

In Venezuela house flies appear to have developed only a slight degree of resistance to DDT in spite of the extensive application of residual sprays over most of the country as a part of the malaria control campaign during the past few years (Upholt 1950). In the Puerto Cabello area, where filariasis is endemic, Gabaldon reports (1951) that there is some field and laboratory evidence which indicates that *Culex fatigans*[2] may be developing resistance to DDT residuals applied to the interiors of houses.

Central America and the Caribbean

In Trinidad, B.W.I., general observations indicate that house flies develop resistance in areas that have been treated with DDT residual sprays for two or more years. *Anopheles aquasalis* has displayed no resistance in spite of the extensive application of DDT residual sprays in many districts for more than 5 years (Gillette 1951).

In Panamá, where there has been considerable use of DDT residual sprays in recent years, the general experience is that DDT, chlordan, and BHC are no longer as effective against house flies as they were several years ago (Trapido 1951A). In some situations very high degrees of resistance have developed, as for example around some garbage landfills where the early effectiveness of DDT resulted in a relaxation of sanitation practices (Smith 1951). After DDT became ineffective, BHC was used with success for several years, but the house flies now exhibit a terrific resistance to DDT, BHC, dieldrin, chlordan, and other currently available insecticides (Ibid.). Needless to say, this has stimulated a return to sanitation. *Anopheles albimanus*, the principal malaria vector in Panamá, and the yellow fever mosquito, *Aedes aegypti*, show no indication of the development of resistance.

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[2] *C. quinquefasciatus* Say.

In Costa Rica a DDT residual spray program was initiated in June, 1950, with applications at 6 month intervals. At the end of the fourth application, there is no indication of resistance in *Anopheles albimanus* (Mendez 1951).

In Mexico DDT residual sprays have been applied in various areas for the control of flies and mosquitoes during the past several years. Professor Hecht (1951) states that in dairy barns around Mexico City where DDT sprays were first applied in 1945, complaints of unsatisfactory results were first received in 1947, and subsequently resistance became so great that this method of control was abandoned. Overall indications are that DDT-resistant house flies are now generally common in Mexico, although Professor Hecht reports that lindane is still effective against the resistant strains with which he has come in contact. Investigations by the Communicable Disease Center have revealed DDT-resistant house flies in the lower Rio Grande area of northern Mexico (Upholt 1950).

In the rice-growing towns in the State of Morelos where *Anopheles pseudopunctipennis* has been exposed to DDT residual sprays since 1945, there is no evidence that the species has developed any resistance (King 1950; Downs *et al.* 1950). *Anopheles aztecus*, the malaria vector in Xochimilco region of the Valley of Mexico is still effectively controlled by DDT residual sprays in areas which have been treated since 1948 (Downs and Bordas 1951).

In Puerto Rico there are indications that house flies in the San Juan area have developed DDT resistance as a result of two heavy applications of residual sprays, DDT is still highly effective in the control of *Anopheles albimanus*, but a high level of resistance is believed to have developed in *Culex* mosquitoes (Pimentel 1951).

Canada

In Canada complaints were received in early 1949 that DDT residuals were ineffective against house flies in certain areas in Ontario and Quebec, and subsequent laboratory tests confirmed the fact that the flies were building up resistance (Roadhouse 1952). At the present time DDT resistance has increased to such an extent in Canada that in many areas this insecticide is of little or no value for fly control. Flies collected from Ontario and Quebec in 1951 had developed DDT resistance averaging 3,000 times that of the laboratory strain. In British Columbia and Alberta resistance appeared in 1951 and was found to be 100 times normal. In general, house fly resistance to DDT in Canada is spotted and there are still many areas where resistant flies have not yet developed.

United States

House flies

Extensive residual applications of DDT were first made in this country in 1945 in connection with the extended malaria control program of the Public

Health Service. Lindquist and Wilson (1948) showed in studies which they initiated in January, 1946, that laboratory strains of house flies could develop resistance to DDT. The first published evidence of resistant field strains of house flies was obtained in early 1948 by Barber and Schmitt (1949) from a hotel in Ellenville, New York, where excellent control of house flies had been achieved with DDT residuals during the two previous seasons.

Numerous complaints of ineffective fly control were received by the Communicable Disease Center during the 1947 season, and some of these were undoubtedly due to the development of DDT resistance (Simmons 1948). Experimental confirmation of the occurrence of DDT resistance in field strains of house flies was first obtained by the Communicable Disease Center in early June of 1948 with flies collected from Albany and Columbus, Georgia (Fay 1948). In the Savannah area, where the first experimental applications of DDT residuals were made in 1944, the less satisfactory fly control experienced in 1947 was probably due to DDT resistance although it was not until the spring of 1949 that widespread occurrence of resistance in the area was confirmed by laboratory tests (Quarterman 1950).

In Florida a few reports of difficulties experienced in controlling house flies were received in 1947, and a serious problem developed in 1948 (King and Gahan 1949). By 1951 essentially the entire fly population appeared to be resistant (King 1951). In the Tennessee Valley, where the TVA first made experimental applications of DDT residuals in 1944 and began extensive use of this material for malaria control in 1945, quite a few complaints of ineffective fly control were received in 1947, and by 1948 such complaints were widespread in the treated areas.

DDT residuals were first applied for fly control in southern California in 1946, and failures of these residuals to control house flies were first observed in the spring of 1948 (March and Metcalf 1950). During the following season field strains were collected which were as much as 300 times as resistant to DDT as the laboratory strain. In Oregon, DDT-resistant flies were observed in 1949 (Roth and Mote 1950). In Illinois, Decker and Bruce (1951) found in a survey of farms in 1949 that over 87 per cent of them had resistant flies, and by 1950 all farms surveyed had resistant flies. Hansens *et al.* reported DDT-resistant house flies from New Jersey in 1948.

Although DDT-resistant house flies are widespread in this country, there are apparently field strains against which DDT is still effective from a practical control standpoint (Upholt 1950). An overall evaluation of the Public Health Service DDT residual spray programs for the 1948, 1949, and 1950 seasons showed that house fly resistance had not become of sufficient importance measurably to affect the degree of fly control obtained during the three year period (Lyman and Bradley 1951). However, in some situations the problem of house fly resistance appears to be increasing in magnitude with each successive year. Resistance seems to be less widespread in the more northern states, and is still quite spotted in the northwestern states in particular (Lindquist 1951).

Although DDT-resistant house flies were initially susceptible to certain other chlorinated hydrocarbons, they rapidly developed resistance to these. For example, March and Metcalf (1950) found that DDT-resistant flies on a poultry farm developed resistance to BHC within a few months after its initial use and dieldrin was also ineffective. Geib (1951) has reported a similar experience in which DDT- and BHC-resistant house flies developed resistance to dieldrin from a single application. Field experiments with dieldrin conducted by the Technical Development Branch of the Communicable Disease Center have shown that effective control of DDT-resistant house flies could be obtained for two full seasons in some areas, whereas in others flies developed a high degree of resistance within two months (Quarterman 1950). High degrees of resistance to chlordan have also developed within a short time after its initial use. Multiple-resistant strains are present in various parts of the country which cannot be controlled with residual or space sprays of DDT, methoxychlor, dieldrin, chlordan, lindane, heptachlor, or other chlorinated hydrocarbons. Among the materials which show some promise for the control of these resistant flies are Dilan as a residual spray, and Dilan, pyrethrum, or DDT-synergist combinations for space sprays; however, there is indication from laboratory tests that resistance to these materials may also develop within a relatively short time. Field strains of blow flies (*Phaenicia* and *Callitroga*) do not appear to have developed any resistance to the chlorinated hydrocarbons. Recent laboratory studies by Fay and his associates of the Communicable Disease Center indicate that *Phaenicia pallescens* does not exhibit a pattern of development of resistance to chlorinated hydrocarbons similar to that observed for *Musca domestica*.

Mosquitoes

Unsatisfactory control of salt-marsh mosquitoes (*Aedes sollicitans* and *A. taeniorhynchus*) with DDT was reported in late 1948, and during the 1949 season in areas of Florida which had been treated on a small scale in 1943 and 1944 and extensively since 1945. Workers from the Orlando laboratory of the Bureau of Entomology and Plant Quarantine showed that these mosquitoes had acquired a high level of resistance to DDT (Knipling 1950). BHC, lindane, dieldrin, and parathion were effective against these resistant strains. Resistance of salt-marsh mosquitoes to DDT larvicides is now widespread in Florida.

In California reports of unsatisfactory control of *Aedes nigromaculis* with DDT larvicides were received in 1948, and this resistance was confirmed experimentally in October 1949 (Smith 1949; Bohart and Murray 1950). Toxaphene gave satisfactory control of this resistant strain. Indications of DDT resistance in *A. dorsalis* during the 1949 season were also reported by Geib (1950). During the 1951 season investigations have shown that *Culex tarsalis* has also developed a marked resistance to DDT as well as several other chlorinated hydrocarbons, including toxaphene, lindane, aldrin, and heptachlor (Gjullin and Peters 1952). It has been shown by laboratory tests that *Anopheles quadrimaculatus* is capable of developing at least a slight resistance to DDT (Fay *et al.* 1949); however, on the basis of inspection of sprayed houses, DDT residual sprays appear still to be highly effective against this species in areas which the Public Health Service has been treating since 1945 (Bradley and Lyman 1950). This does not necessarily indicate that a behavioristic type of resistance has not developed such as has been indicated for *A. gambiae* in Africa and *A. darlingi* in South America. This is borne out by recent observations by Ludvik *et al.* (1951) in the Tennessee Valley which indicate marked changes in the reaction pattern of *A. quadrimaculatus* to DDT residuals in comparison with observations made in that area in 1944 (Metcalf *et al.* 1945). He also obtained some suggestion of physiological resistance to DDT in populations where this insecticide had been routinely used for the past five years though the results were not conclusive. Studies by Quarterman *et al.* (1949) in the Savannah, Georgia, area indicated a repellent action of DDT residuals against *A. quadrimaculatus*, catches of mosquitoes being 5 times greater in untreated than in treated animal-baited houses.

Other Insects

There appears to be some evidence of the development of resistance to DDT by the Oriental rat flea, *Xenopsylla cheopis*, in the last year or two, but this is still rather indefinite; in any case, such resistance as may have developed is not sufficient seriously to effect operational programs where 10 per cent DDT dusts are used to control this species (Good 1951). Studies by Kilpatrick and Fay (1951) have shown that this species can develop resistance to DDT under laboratory conditions.

There are scattered references to the development of resistance by other insect pests of man which may be of potential importance in disease transmission. Johnson and Hill (1948) have reported partial resistance of a strain of bedbugs to DDT residuals. Grayson (1951) has recently shown that the German cockroach (*Blatella germanica*) can develop resistance to DDT under laboratory conditions. DDT-resistant filter flies (*Psychoda alternata*) were reported from Illinois in 1949 (Bruce 1950).

DISCUSSION

From an overall analysis of field experiences with insecticide resistance in various parts of the world, there are certain generalizations which are worth summarizing for their implications with reference to future programs.

Rates of Development of Resistance

The rate at which insecticide resistance develops in field programs under extensive use of insecticides shows considerable variation which may be due to genetic differences, climatic factors, extent and intensity of treatment, or other unknown causes. House flies have developed DDT resistance as soon as the first season of use and as late as the sixth season, but in most situations significant resistance has developed during the second or third year of extensive use. DDT-resistant flies have usually developed resistance to BHC in less than a year and sometimes within a few weeks, although in at least one instance BHC effectively controlled DDT-resistant house flies for several years. In Egypt, house flies which had never been exposed to DDT, developed resistance to BHC within a year. DDT-resistant house flies have also usually developed resistance to chlordan or dieldrin in less than a season, but both of these materials have given effective control for two full seasons in some situations. Field strains with multiple resistance to chlorinated hydrocarbons either already possess or develop almost immediately a resistance to other chlorinated hydrocarbons.

Resistant salt-marsh mosquitoes (*Aedes sollicitans* and *A. taeniorhynchus*) were observed in Florida at the end of the sixth season after initial use and the fourth season after extensive use of DDT. In California, DDT-resistant *Aedes nigromaculis* and *Culex tarsalis* seem to have appeared as soon as the third or fourth season of extensive use. Resistance to toxaphene appeared in the second or third season of use, and resistance to aldrin after one season of use. *Anopheles sacharovi* in Greece began to show signs of DDT resistance during the sixth season of extensive use, as did *Pulex irritans*. The first signs of DDT resistance in *A. quadrimaculatus* in the Tennessee Valley also appeared about the sixth season of extensive use.

Specificity and Multiple-Resistance

There is general agreement that DDT-resistant house flies are not initially cross-resistant to other chlorinated hydrocarbons except to closely related materials such as methoxychlor and TDE; however, they rapidly develop resistance to other chlorinated hydrocarbons. House fly strains which have developed resistance to two or more chlorinated hydrocarbons are for practical purposes usually immediately resistant to other chlorinated hydrocarbons. The cross-resistance of flies to closely related chemicals is also borne out by the fact that chlordan-resistant strains are usually resistant to dieldrin, and vice versa.

There are many puzzling variations in the picture of cross-resistance in house flies. There are several reports that methoxychlor-resistant flies are not resistant to DDT, whereas DDT-resistant flies are resistant to methoxychlor (Babers and Pratt 1951). A DDT-resistant field strain of house flies collected from an area in Italy where only DDT had been used were susceptible to other chlorinated hydrocarbons and lethane, but were resistant to pyrethrum; however, the DDT-chlordan-resistant strain from Sardinia was susceptible to pyrethrum (Busvine 1951). Pyrethrum-resistant strains have been produced in the laboratory from a non-resistant Italian strain, and this pyrethrum-resistant strain was also more resistant to DDT than the normal laboratory strain (Harrison 1950). In general, chlorinated hydrocarbon-resistant strains are not resistant to unrelated compounds such as pyrethrum, allethrin, and parathion. However, there are cases of such cross resistance in laboratory studies, as for example the report of Wilson and Gahan (1948) that DDT-resistant house flies also showed increased resistance to pyrethrins, rotenone, and thanite. Decker and Bruce (1951) have given an excellent summary of laboratory studies on cross resistance and present information which is of great significance in planning field programs.

From such information as is available, it appears that field patterns of cross resistance in mosquitoes are quite similar to those experienced with flies.

Reversion of Resistant Strains

Laboratory experiences with the reversion of DDT-resistant house flies have been variable, but in most instances field resistant strains which have been reared in the laboratory for successive generations in the absence of DDT have shown a gradual reversion to susceptibility (Harrison 1950; King 1950; Pimentel *et al.* 1950; Missiroli 1951; March and Metcalf 1950; Upholt 1950). In contrast, field experiences give little evidence of any significant degree of reversion. This difference in field and laboratory experiences may be due to the continued maintenance of sublethal residuals through the widespread agricultural and household use of DDT and the persistence of DDT residuals over a number of years. The latter is further borne out by the fact that Weir (1951) observed an apparent partial reversion of a strain of flies resistant to BHC, which is more volatile and shorter lasting than DDT.

Types of Resistance

There is rather general agreement that the development of true physiological resistance is the major factor in the failure of DDT to effect continuing control of house flies. A notable exception is the observation by Wiesmann (1947) that the naturally resistant strain of houseflies which he collected in Sweden showed morphological differences, including more heavily pigmented extremities, stiffer tarsal hair tufts, larger tarsal joints, and thicker pulvilli and articular membranes. D'Alesandro *et al.* (1949) also reported differences in the pulvilli of normal and resistant flies. March and Metcalf (1950) were not able to find morphological differences in a resistant strain from California, and demonstrated by injection techniques that the resistance of this strain was primarily physiological.

There are a number of observations of behavioristic changes in DDT-resistant house flies. The tendency for resistant flies to rest more on untreated horizontal surfaces than on treated walls has been noted in our own observations as well as by many other workers (Bruce and Decker 1950; King and Gahan 1949; Trapido 1951; Alvarado 1951). Decker and Bruce (1951) have also observed resistant house flies which had adopted the stable fly habit of sitting still in one spot, thus lessening the degree of exposure to DDT residuals. There have also been observations that resistant strains might be irritated or knocked down sooner than normal strains, thus preventing their exposure to lethal doses (Barber and Schmitt, 1949; Busvine, 1951). There are common examples of behavioristic resistance in agricultural insect pests (Morrison 1951), and this type of resistance may have received too little attention in house flies; however, at the present time the conquering of physiological resistance appears to be of primary importance in field programs for the control of house flies.

With pest mosquitoes, as with house flies, physiological resistance appears to be the problem of major importance. However, in the case of anopheline mosquitoes, behavioristic resistance may be equally important. Indications of the development of protective patterns of behavior by *Anopheles gambiae*, *A. darlingi*, and *A. quadrimaculatus* have previously been discussed. Even in the case of pest mosquitoes, some workers are of the opinion that changing behavior patterns are important in the development of resistance (Stutz 1951).

Species Differences in Susceptibility

The importance of natural variations in the susceptibility of different species to insecticides is frequently overlooked. For example, *Anopheles claviger* is much less susceptible to DDT than is *A. quadrimaculatus* (Trapido 1951). Natural differences have been noted in the susceptibility to DDT of the two common salt-marsh mosquitoes, *Aedes sollicitans* and *A. taeniorhynchus* (Smith 1951; Stutz 1951). The six mosquito species of most economic importance in California have been shown to vary greatly in their susceptibility to individual insecticides (Bohart 1950).

Species differences may be a factor in the failure of *Musca nebulo* to develop DDT resistance in India as *M. domestica* has done in other regions. General observations with house flies indicate that there is considerable natural variation in the degree of susceptibility to DDT of different field strains. The occurrence of naturally resistant field strains is also borne out by the experiences of Wiesmann (1951) in Sweden.

Importance of Operational Practices

Operational practices appear to be quite important in connection with insecticide resistance, particularly with reference to the rate at which it develops and the degree to which it is maintained. Extensive or intensive routine application of insecticides seems to favor the development of resistance more than spotted casual treatments. Widescale indiscriminate use and faulty applications of insecticides resulting in sublethal residual deposits appear to be important, especially in the maintenance of established levels of resistance in field strains. In general, purely transitory space sprays are less likely to develop resistance than are long-lasting residuals. For example, DDT space sprays gave satisfactory control of house flies in an Oregon dairy barn for 5 years without the development of resistance, whereas application of DDT residual sprays to a hog barn during the same period produced resistant house flies (Morrison *et al.* 1950). Formulations and dosages which are used may also have a bearing upon the rate and extent of development of resistance. The simultaneous use of a material both as an adulticide and a larvicide seems to accelerate the development of resistance (Decker and Bruce 1951).

Effects of Resistance Upon Life History and Vigor

Our own observations, as well as those of many other workers, indicate that field resistant house flies frequently are more vigorous and reappear in fantastic numbers (Missiroli 1951; Weir 1951; Trapido 1951). The fact that fly indices in the Communicable Disease Center residual spray program were greater in 1949 and 1950 than in 1948 (Lyman and Bradley 1951) may possibly have been due to the increased biotic potential of resistant strains. Wiesmann (1947) observed that the resistant strain of house flies he collected from Sweden was more resistant to high and low extremes of temperature. Similar observations have been made by Weir (1951) in Egypt where the greater resistance to adverse temperatures may explain the continued rise in resistant fly populations during the winter when normal populations are low.

Pimentel *et al.* (1951) found that resistant strains of house flies in New York had a longer larval period than non-resistant strains and that the increase was proportionate to the degree of resistance. However, observations of the resistant Bellflower strain in California by March and Metcalf (1950) showed no differences in length of life cycle, average weight, or susceptibility to heat and cold.

Outlook for the Future

Unless entirely new approaches are developed in the very near future, insecticide resistance will bring an end to the DDT era in fly control. In the long run this may be a blessing. Almost every one now recognizes that a return to sanitation is one of the essentials in any permanent solution to the fly problem. Fly-proofing is also receiving the renewed emphasis which it deserves. A similar situation is developing with regard to mosquito control, with mosquito proofing, elimination of breeding areas, and other of the more permanent measures again receiving attention. This return to the basic concepts which prevailed before the DDT era is most desirable, but that in itself is not the answer—we must go forward, not backward. No one who has observed or even read about the miracles in disease control which have been brought about through the use of DDT and other new insecticides in the last decade would want to give up this wonderful new tool (Simmons and Upholt 1951; Pampana 1951). We should by all means attempt to regain the usefulness of these new materials and put them in their rightful place as valuable supplements to more permanent or naturalistic control measures. This may be accomplished in part by the prudent use of information now at hand, by giving better technical guidance to control programs, and making more thorough and careful applications. Space sprays will undoubtedly find increased usage, and some of the recently-developed synergists will certainly increase insecticidal effectiveness against resistant strains. Some new materials, such as Dilan, offer temporary promise as residuals, but it is inevitable that resistance will also develop to these new materials if utilized in the same old way (Decker and Bruce 1951).

The real solution to the insecticide resistance problem lies in a return to fundamental research. We need to do much more research on the general physiology of insects, on the modes of action of insecticides, and on the mechanism and genetic basis of resistance. We need also to return to basic studies on the biology and habits of insects. This type of research has been sorely neglected during the DDT era when many seemed to believe that the only knowledge one needed in order to control insects was the operational procedures for a DDT residual spray program. If we refill our reservoirs of basic knowledge, we may find ways of blocking the detoxification process, new insecticides which may irreversibly affect a vital physiological process, complementary materials with paired alternate actions, or other new approaches based on sound knowledge rather than blind screening.

Just as surely as the social world needs a return to religion, so does the scientific world need a return to fundamentals!

REFERENCES

Aitken, Thomas H. G. 1951, Personal communication of November 14, 1951.

Alvarado, Carlos A. 1951, Personal communication of November 14, 1951.

Babers, Frank H., and **John** J. **Pratt, Jr.** 1951, Development of insect resistance to insecticides—II. A critical review of the literature up to 1951, *U.S.D.A., B.E.P.Q.*, E-818.

Barber, George W., and **John** B. **Schmitt** 1948, House flies resistant to DDT residual sprays, *New Jersey Agr. Exp. Sta. Bull.* 742, August 1948.

Barber, George W., and **John** B. **Schmitt** 1949, Further studies on resistance to DDT in the housefly, *J. Econ. Entomol.* **42**(2): 287–292.

Barnard, Chester I. 1949, Malaria in Sardinia, Rockefeller Foundation "A Review for 1949" pp. 35–40.

Bettini, S., and B. **Barachini** 1948, Primi resultati della lotta con l'Octa-Klor ed il gammaesano contro le mosche domestiche resistenti al DDT, *Riv. di Parassitol.* **9**(2): 85–91.

Bohart, Richard M. 1950, Laboratory studies of mosquito larvicides, *Proc. Joint Meetings Am. Mosq. Cont. Assoc. and Va. Mosq. Cont. Assoc.* pp. 72–74, February 1950.

Bohart, Richard M. and W. D. **Murray** 1950, DDT resistance in *Aedes nigromaculis*, *Proc. 18th Ann. Conf. of Calif. Mosq. Cont. Assoc.* pp. 20–21.

Bradley, G. H., and F. E. **Lyman** 1950, Discussion of five years' use of DDT residuals against *Anopheles quadrimaculatus*, *J. Nat. Mal. Soc.* **9**(2): 113–118.

Brown, A. W. A. 1951, The development of resistance of insects to insecticides, *81st An. Rept. Ent. Soc. of Ontario* 1950, pp. 34–35.

Brown, A. W. A. 1951A, Personal communication of September 20, 1951.

Bruce, W. N. 1950, Current report on housefly resistance to insecticides, *Pest Control* **18**(4): 9–10, and 19.

Bruce, W. N., and G. C. **Decker** 1950, House fly tolerance for insecticides, *Soap Sanit. Chem.* **26**(3): 122–125, 145–147.

Bushland, R. C., L. C. **McAllister, Jr., Howard** A. **Jones,** and G. H. **Culpepper** 1945, DDT powder for the control of lice attacking man, *J. Econ. Entomol.* **38**(2): 210–217.

deBustamante, Fernando M., **Olympio da Silva Pinto, Arino** S. **Guedes.** S. H. **Xavier,** and J. R. **Freitas** 1951, Sobre a captura do *Anopheles darlingi* e *Anopheles albitarsis* nas paredes externas de cases dedetizadas em Engenheiro Dolabela, Minas Gerais, *Rev. Brasil, Malar. e Doenças Trop.* **3**(1): 122–129 (As summarized *Mosq. News* **11**(3): 186, September 1951.)

Busvine, J. R. 1949, A test for DDT-resistant flies, *Ceylon J. Med. Science*, Sec. D **6**(4): 1–3.

Busvine, J. R. 1951, Mechanism of resistance to insecticides in house flies, *Nature* **168**: 193.

Busvine, J. R. 1951A, Personal communication of November 19, 1951.

D'Alessandro, G., G. **Catalano,** M. **Mariani,** E. **Scerrino, Calogera Smiraglia,** and G. **Valguarnera** 1949, Sulla resistenza delle mosche al DDT, *Sicilia Medica* **6**: 5–16.

Decker, George C., and W. N. **Bruce** 1951, Where are we going with fly resistance? *Soap Sanit. Chem.* **27**(6): 139–143, 159.

Downs, W. G., and E. **Bordas** 1951, *Anopheles aztecus*, malaria, and malaria control in the Valley of Mexico, *J. Nat. Mal. Soc.* **10**(4): 350–358.

Downs, W. G., **Heliodoro Celis** S., and **James** B. **Gahan** 1950, Control of *Anopheles pseudopunctipennis* in Mexico with DDT residual sprays applied in building. III. Malariological observations after five years of spraying, *Am. J. Hyg.* **52**(3): 348–352.

Eddy, G. W. 1951, A report on the effectiveness of certain insecticides against DDT resistant body lice in Korea. Paper presented at annual meeting of *Amer. Assoc. of Ec. Ent.*, Cincinnati, December 1951.

Fay, R. W. 1948, Second report on studies of Columbus and Albany house flies. Unpublished report submitted to Technical Development Services, CDC, June 1948.

Fay, R. W., Walter C. Baker, and Mary M. Grainger 1949, Laboratory studies on the resistance of *Anopheles quadrimaculatus* to DDT and other insecticides, *J. Nat. Mal. Soc.* **8**(2): 137–146.

Gabaldon, Arnoldo 1951, Personal communication of September 27, 1951.

Gahan, James B., and John M. Weir 1950, Houseflies resistant to benzene hexachloride, *Science* **111**: 651.

Geib, A. F. 1950, An indication of mosquito larvae resistance to DDT, *Proc. Joint Meetings Am. Mosq. Cont. Assoc. and Virg. Mosq. Cont. Assoc.* Feb. 1950, pp. 68–71.

Geib, A. F. 1951, Personal communication of September 22, 1951.

Gillette, Horace 1951, Personal communication of December 15, 1951.

Gjullin, C. M., and R. F. Peters 1952, The present status of mosquito resistance to insecticides in California, In press, *Mosquito News* 1952.

Good, Newell E. 1951, Personal communication of November 21, 1951.

Grayson, J. M. 1951, Reponse of the German cockroach to sublethal concentrations of DDT and benzene hexachloride. Presented at annual meeting of *Am. Assoc. Ec. Ent.* December 12, 1951.

Hadaway, A. B. 1950, Observations on mosquito behaviour in Native Huts, *Bull. Entomol. Res.* **41**(1): 63–78.

Hadjinicolaou, John 1951, Personal communication of October 10, 1951.

Hansens, Elton J., John B. Schmitt, and George W. Barber 1948, Resistance of house flies to residual applications of DDT in New Jersey, *J. Econ. Entomol.* **41**(5): 802–803.

Harrison, C. Mary 1950, DDT-Resistant houseflies, *Annals of Applied Biology* **37**(2): 306–309.

Hecht, Otto 1951, Personal communication of December 21, 1951.

Hurlbut, Herbert S., Robert M. Altman, and Carlyle Nibley, Jr. 1952, DDT resistance in Korean body lice, *Science* **115**(2875): 11–12.

Johnson, M. S., and A. J. Hill 1948, Partial resistance of a strain of bedbug to DDT residual, *Medical News Letter* (Bur. Med. & Surg. Wash.) **12**(1): 27–28.

Keiding, J., and H. Van Deurs 1949, DDT resistance in houseflies in Denmark, *Nature* **163** (4155): 964–965.

Kilpatrick, John W., and Richard W. Fay 1951, DDT-resistance studies with the Oriental rat flea, *Xenopsylla cheopis* (Roth). In press, *J. Econ. Entomol.*

King, W. V. 1951, Personal communication of September 12, 1951.

King, W. V., and J. B. Gahan 1949, Failure of DDT to control house flies, *J. Econ. Entomol.* **42**(3): 405.

King, W. V. 1950, DDT-resistant house flies and mosquitoes, *J. Econ. Entomol.* **43**(4): 527–532.

Knipling, E. F. 1950, Insecticidal resistant flies and mosquitoes, *Proc. Chem. Spec. Manu. Assoc.* pp. 87–88, June 1950.

Lindquist, A. W. 1951, Personal communication of September 10, 1951.

Lindquist, A. W., and H. G. Wilson 1948, Development of a strain of house flies resistant to DDT, *Science* **107** (2776): 276.

Logan, John A. 1950, *Anopheles labranchiae* eradication in Sardinia: an interim report, *Am. J. Trop. Med.* **30**(2): 313–323.

Ludvik, G. F., W. E. Snow, and W. B. Hawkins 1951, The susceptibility of *Anopheles quadrimaculatus* to DDT after five years of routine treatment in the Tennessee River Valley, *J. Natl. Mal. Soc.* **10**(1): 35–43.

Lyman, F. Earle, and George H. Bradley 1951, Fly control incidental to the residual spray program, *CDC Bull.* **10**(8): 19–21.

Mackerras, I. M. 1951, Personal communication of November 30, 1951.

March, Ralph B., and R. L. Metcalf 1950, Studies in California of insecticide-resistant flies, *Soap and Sanit. Chem.* CSMA Proc. pp. 80–83, June 1950.

Melander, A. L. 1914, Can insects become resistant to sprays? *J. Econ. Entomol.* **7**(2): 167–172.

Mendez, O. Vargas 1951, Personal communication of October 5, 1951.

Metcalf, R. L., A. D. Hess, G. E. Smith, G. M. Jeffery, and G. L. Ludwig 1945, Observations on the use of DDT for the control of *Anopheles quadrimaculatus*, *Pub. Hlth. Reports* **60**(27): 753–774.

Missiroli, A. 1950, The control of domestic insects in Italy, *Amer. J. Trop. Med.* **30**(5): 773–783.

Missiroli, A. 1951, Resistenza agli insecticide di alcune razze di *Musca domestica*, *Riv. Parassit.* **12**(1): 5–25.

Morrison, Frank O. 1951, Morphological and habit changes correlated with resistance to insecticidal treatment, *81st Ann. Rpt. of Entomol. Soc. of Ontario* 1950, pp. 41–43.

Morrison, H. E., R. W. Lauderdale, H. H. Crowell, and Don C. Mote 1950, Space spraying for fly control in dairy barns, *J. Econ. Entomol.* **43**(6): 846–850.

Mosna, E. 1949, Octa-Klor, gammaesano, e toxaphene usati contro le mosche DDT-resistenti, *Riv. di Parassitol.* **10**(1): 31–51.

Mosna, E. 1947, Su una carratteristica biologica del *Culex pipiens autogenicus* di Latina, *Riv. Parassit.* **8**(23): 125–126.

Mosna, E. 1950, Il controllo con Octa-Klor delle mosche domestiche DDT-resistenti, *Riv. di Parassitol.* **11**: 27–35.

Pal, Rajindar 1951, Personal communication of October 1, 1951.

Pal, Rajindar 1951A, Report on the Ninth International Congress in Entomology, December 4, 1951.

Pampana, E. J. 1951, Lutte antipaludique par les insecticides a action remanente: Resultes des grandes campagnes, *Bul. W.H.O.* **3**(4): 557–614.

Pimentel, David 1951, Personal communication of November 11, 1951.

Pimentel, David, H. H. Schwardt, J. E. Dewey, L. B. Norton 1950, Studies on insecticide resistant house flies in New York, *Soap Sanit. Chem.* CSMA Proc., pp. 94–96, December 1950.

Pimental, David, J. E. Dewey, and H. H. Schwardt 1951, An increase in the duration of the life cycle of DDT-resistant strains of the house fly, *J. Econ. Entomol.* **44**(4): 477–481.

Pinotti, Marion 1951, Personal communication of November 19, 1951.

Quarterman, K. D. 1950, The status of fly resistance to insecticides in the Savannah area and its implications in the general problem of fly control, *CDC Bull.* **9**(11): 3–7.

Quarterman, K. D., J. Jensen, W. C. Baker, and W. Mathis 1949, Investigations on the effects of DDT residual treatments on the resting and feeding habits of mosquitoes, *T.D.D. Summary of Activities* No. 16, January 28, 1949.

Reed, W. D. 1952, Personal communication of January 5, 1952.

Roadhouse, L. 1952, Laboratory studies of DDT-resistant house flies in Canada. In press, *Canadian Entomologist* 1952.

Roth, A. R., and Don C. Mote 1950, DDT-resistant flies in Oregon, *J. Econ. Entomol.* **43**(6): 937–938.

Russell, Raymond 1949, Control de moscas de establo, *Agronomia* (Peru) **14**(58): 43–45.

Sacca, G. 1947, Sull'esistenza di mosche domestiche resistenti al DDT, *Riv. Parassit.* **8**(2–3): 127–128.

Simmons, S. W. 1948, Personal communication of June 28, 1948.

Simmons, S. W., and W. M. Upholt 1951, Disease control with insecticides, *Bull. World Hlth. Org.* **3**: 535–556.

Singh, Jaswant 1951, Personal communication of September 12, 1951.

Smith, G. 1949, Kern MAD has circumstantial evidence on DDT resistance, *Mosq. Buzz* **3**(8): 2.

Smith, Palmer 1951, Personal communication of September 27, 1951.

Stutz, Fred H. 1951, Personal communication of September 26, 1951.

Thomson, R. C. Muirhead 1947, The effects of house spraying with pyrethrum and with DDT on *Anopheles gambiae* and *A. melas* in West Africa, *Bull. Ent. Res.* **38**(3): 449–464.

Thomson, R. C. Muirhead 1947A, Recent knowledge about malaria vectors in West Africa and their control, *Trans. R. Soc. Trop. Med. Hyg.* **40**(5): 511–527.

Trapido, Harold 1951, The toxicity of DDT to *Anopheles claviger* (Meigen) in Sardinia and on the Italian mainland, *J. Nat. Mal. Soc.* **10**(3): 266–271.

Trapido, Harold 1951A, Personal communication of September 10, 1951.

Upholt, W. M. 1950, Significance to the insecticide industry of fly resistance, *Soap Sanit. Chem.* CSMA Proc. pp. 69–71, December 1950.

Villalobos, C. Enrique 1951, Personal communication of October 9, 1951.

Weir, John M. 1951, Personal communications of September 25, and December 5, 1951.

Waterhouse, D. F. 1951, Personal communication of December 7, 1951.

Wiesmann, R. von 1947, Untersuchungen über das physiologische Verhalten von *Musca domestica* L. verschiedener Provenienzen, *Schweiz. Ent. Gesell. Mitt.* **20**: 484–504.

Wiesmann, R. von 1952, Personal communication of February 10.

Wilson, H. F., and J. B. Gahan 1948, Susceptibility of DDT-resistant houseflies to other insecticidal sprays, *Science* **107**(2776): 276–277.

PRESENT STATUS OF MOSQUITO RESISTANCE TO INSECTICIDES

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The appearance of house flies resistant to DDT in 1947 and 1948 led to speculation as to whether the resistance problem would become significant in the control of mosquitoes and other insects of medical importance. Mosna (1947, 1948) reported the occurrence of resistant strains of *Culex pipiens autogenicus* Roub. in Italy as early as 1947, which apparently resulted from the segregation of resistant individuals where an intensive house-spraying program was being carried out.

The importance of the resistance problem in mosquito control in the United States was fully apparent in 1949 in Florida, where the salt-marsh mosquitoes *Aedes taeniorhynchus* (Wied.) and *A. sollicitans* (Wlkr.) predominate During that year DDT was not effectively controlling these species in several areas in the State. In fact, failures to obtain satisfactory control of mosquitoes with DDT were reported in some areas late in 1948. The Orlando, Florida, laboratory of the Bureau of Entomology and Plant Quarantine, which is conducting research on insect problems of importance to the armed services with funds provided by the Department of Defense, investigated the problem in 1949 in cooperation with the Florida mosquito abatement districts and several branches of the Department of Defense. Laboratory and field tests soon established that salt-marsh mosquitoes in Brevard County had acquired a high resistance to DDT. Results of these investigations were reported by Deonier and Gilbert (1950) and King (1950). Comparison of larvae and adults collected from both treated and untreated areas showed that some local strains were approximately ten times as resistant to DDT as the susceptible strains. We have not encountered the high resistance among mosquitoes that occurs among flies. However, it is impractical to attempt widespread mosquito control with DDT when the rate of application has to be increased from five to ten times that required for susceptible strains.

In 1949 workers in California also reported failure of DDT to control certain mosquitoes in some of the areas in that State. Field observations and controlled laboratory tests reported by Bohart and Murray (1950) showed that resistance was responsible for the difficulties.

CULICINE MOSQUITOES

In 1950 and 1951 the resistance problem with culicine mosquitoes became progressively more acute in Florida and California. Most of the mosquito-abatement districts in Florida are now having difficulty in controlling salt-marsh mosquitoes with DDT. Repeated tests with larvae collected in treated and non-treated areas have shown that DDT-resistant mosquitoes are widespread in the State. The degree of resistance varies greatly among different lots of larvae. As in the case of flies, the mosquitoes resistant to DDT are also resistant to the related insecticide TDE, but they show little or no resistance to lindane, benzene hexachloride, toxaphene, dieldrin, or chlordan where these materials have not been employed for control.

Field experiments by Deonier *et al.* (1950) demonstrated that benzene hexachloride was the best substitute for DDT for controlling adults of DDT-resistant strains. This insecticide was therefore used in control operations in Florida during 1950 and 1951. Results in general have been satisfactory. However, there are indications that mosquitoes in some areas are becoming more difficult to control with this insecticide after two seasons' use.

Many substitute insecticides have been tested by J. C. Keller and his associates of the Orlando laboratory (unpublished data). Following the report by Sumerford *et al.* (1951) that Dimite (or DMC) in combination with DDT increased the effectiveness of DDT against resistant house flies, this and several thousand other compounds were tested at Orlando in combination with DDT. Although some of the materials exert some synergistic action, none of those thus far tested seem to offer a solution to the problem.

Studies on mosquito resistance were carried out in California during the current (1951) season. These investigations were conducted jointly by the Bureau of Vector Control, the California Department of Health, and the Bureau of Entomology and Plant Quarantine. The work was done by C. M. Gjullin, of the Corvallis laboratory, and by personnel assigned by the Bureau of Vector Control and various mosquito abatement districts in the San Joaquin Valley. The details of these studies are being published elsewhere (Gjullin and Peters 1952), but a brief summary of their findings is reported at this time.

The studies were carried out in the San Joaquin Valley, principally in areas around Bakersfield, Visalia, Fresno, and Merced. DDT and other chlorinated hydrocarbon insecticides have been used extensively in California since 1946. The objective of the study was to determine whether the larvae of the more common species of mosquitoes were resistant to DDT, toxaphene, lindane, aldrin, and certain other insecticides. The approximate dosage giving 50 per cent mortality was established for larvae collected from areas where insecticides had been employed for mosquito control for several years and from areas where insecticides have not been used.

It was found that larvae of *Aedes nigromaculis* (Ludlow) showed resistance to DDT in all areas where the insecticide had been used in recent years. The degree of resistance ranged from 2 to 11 times that for larvae from untreated areas. In some tests *Aedes dorsalis* (Meig.) showed a similar trend. In general, larvae of *A. nigromaculis* showed no resistance to toxaphene, lindane, and aldrin, although slight resistance was indicated in some areas.

Culex tarsalis Coq. also showed marked resistance to DDT in the same areas. The larvae were five to ten times as resistant as those taken from untreated areas and considered to be normally susceptible. In most areas the *C. tarsalis* larvae resistant to DDT showed no resistance to other insecticides. However, the larvae in a duck-club area in Kern County acquired a high resistance to several materials.

DDT was used as a larvicide in this area from 1946 to 1948. Toxaphene was employed in 1949 and DDT, toxaphene and aldrin in 1950.

In August 1951 larvae of *C. tarsalis* from one of the duck clubs, in comparison with susceptible larvae, were 10 times as resistant to DDT, 33 times as resistant to toxaphene, 11 times as resistant to lindane, 215 times as resistant to aldrin, and 1300 times as resistant to heptachlor. In this situation, therefore, this species has acquired a high degree of resistance not only to insecticides that have been employed but to certain related insecticides that have not been used before. Thus, we have a development which parallels the resistance problem among house flies.

What is the current situation among culicine mosquitoes in states other than Florida and California? I asked a number of mosquito research and control organizations whether they had found resistance among pest mosquitoes. According to D. M. Rees, resistance has not become apparent in the Salt Lake City, Utah, mosquito-control district. B. B. Pepper reported no evidence of resistance in various districts in New Jersey. R. E. Dorer, engineer in charge of the mosquito work in the vicinity of Norfolk, Virginia, reported an apparent decrease in efficacy of aerial spraying at Langley Field. Special studies conducted by the Corvallis, Oregon, laboratory in 1950 indicated that resistance had not developed in the Portland, Oregon, area.

ANOPHELINE MOSQUITOES

The situation in regard to insecticide resistance among anopheline mosquitoes appears to be more favorable at the present time. Gahan *et al.* (1949) found no evidence of resistance of adult *Anopheles pseudopunctipennis* (Theob.) to DDT residues following four years' use of residual treatments in an isolated village in Mexico. No reports have been issued on the resistance to DDT resulting from the extensive house-spraying operations in connection with the malaria control program conducted by the Public Health Service and the cooperating States. In laboratory studies Fay *et al.* (1949) found that exposure of adults of *A. quadrimaculatus* (Say) through several generations to residues of DDT caused only slight resistance to the insecticide. Trapido (1951) found that larvae of *A. claviger* (Meig.) in Sardinia showed no resistance to DDT after four years' intensive use of DDT as a residual treatment and as a larvicide.

O. M. Derryberry, Director of Health, Tennessee Valley Authority, stated in personal communications that their data were inconclusive as to the presence of resistance among *A. quadrimaculatus* after extensive use of DDT as a larvicide and as a residual spray in the Tennessee Valley.

RELATIONSHIP BETWEEN MOSQUITO-CONTROL PRACTICES AND RESISTANCE

Bruce (1950) has shown that flies exposed through successive generations both in the larval and adult stages, will develop resistance to insecticides more quickly and to a higher degree than when adults only are exposed. It is probable that the same will be true for mosquitoes.

Brevard County was the first area in Florida where salt-marsh mosquitoes developed a high degree of resistance to DDT. This was the area where the Orlando laboratory conducted early experimental work with DDT. As early as 1943 DDT was applied on large plots to both adults and larvae. By 1944 the Navy was applying DDT routinely to hundreds of acres at Banana River where mosquitoes were abundant. The sprays were applied to areas where adults concentrate as well as to marshes where larvae develop. Both stages of the insects were therefore exposed to the insecticide. DDT treatments in Brevard County from 1943 to 1949 resulted in the possible accumulation of 10 pounds or more of DDT per acre. Other mosquito districts in Florida also have employed DDT during recent years on extensive marsh areas and others where adults concentrate.

From studies on prehatching treatments in various mosquito breeding areas, we know that the application of as little as 0.5 pound of DDT per acre can exert a toxic effect on larvae for several years. Every brood of salt-marsh mosquito larvae in these Florida marshes was therefore exposed to the DDT residues for several years, even though specific larvicidal treatments were not applied to some broods. Since other large areas where adults concentrated were also treated repeatedly, the exposure of mosquitoes in certain areas in Florida approximates the dual larval and adult exposure to insecticides given house flies under laboratory conditions by Bruce of Illinois.

A similar situation existed in California, in that specific treatments of larvae and adults as well as the residual action of previous larvicidal treatments undoubtedly resulted in almost continuous exposure of mosquitoes to insecticides in some of the irrigated sections.

Where species of mosquitoes occur that are known to be capable of becoming resistant to insecticides, serious thought should be given to changes in control practices which may prevent, or at least retard, the development of resistance.

Wherever practical, we should emphasize environmental control of larvae through drainage, filling, and other similar measures. It may also be best to employ insecticides for control of adults only, wherever feasible. Furthermore, it may be more advantageous from the standpoint of resistance, as well as economy, to restrict the use of insecticides to the immediate area where control of adults is needed. This might be done by applying minimum amounts of spray or aerosol as often as necessary to keep mosquitoes under control. A second method which offers considerable promise is to apply residual treatments to vegetation and other resting places in and around homes, resort areas, camps, etc. Studies by the Corvallis laboratory with DDT against snow-water mosquitoes and by the Orlando laboratory with DDT and benzene hexachloride against salt-marsh species indicate that a marked reduction of adult mosquitoes can be obtained during daylight hours for a week or more by applying residual insecticides at the rate of 1 to 2 pounds per acre to areas of only a few acres.

NEED FOR ADDITIONAL RESEARCH

Those responsible for research and control of mosquitoes are in general agreement that we should not relax our efforts to develop new materials and methods for the control of mosquitoes. There is every reason to believe that the resistance problem will become more widespread among culicine mosquitoes. Although the situation with respect to anopheline mosquitoes does not seem alarming at present, it would be unwise to assume that resistance will not develop in time among some or perhaps all species of *Anopheles* subjected to widespread and intensive control with insecticides.

It is my opinion that the search for new mosquito insecticides should continue. Perhaps new materials may be found to which mosquitoes cannot develop a high degree of resistance. The possibilities for synergists to nullify the resistance should be explored further. New approaches to mosquito control which do not involve the use of insecticides should be investigated. However, basic studies to learn more about the physiology of nonresistant and resistant insects and the mode of action of insecticides should be expanded.

The widespread occurrence of resistant house flies, the appearance of at least six species of culicine mosquitoes resistant to specific insecticides in localized areas, and the occurrence of body lice highly resistant to DDT in the Far East fully emphasize the significance of the resistance problem in the field of medical entomology. The discovery of resistant lice in Korea was only recently reported by military personnel and confirmed by members of the Orlando laboratory sent on a special mission to Korea by the Army Committee on Insect and Rodent Control.

The fact that certain species among the three most important groups of medically important arthropods, have developed almost complete immunity to the insecticides formerly considered practical and permanent means for their control, should provide ample justification for securing the best available scientific talent in an effort to find a solution to the resistance problem.

SUMMARY

Several species of culicine mosquitoes, including *Culex pipiens autogenicus*, *C. tarsalis*, *Aedes taeniorhynchus*, *A. sollicitans*, *A. nigromaculis*, and *A. dorsalis*, have developed resistance to DDT in localized areas. This resistance is sufficiently high to make control measures with DDT impractical. Moreover, some of the species have developed resistance to substitute insecticides used for their control, including toxaphene, benzene hexachloride and aldrin.

Resistance to DDT among natural populations of *Anopheles* mosquitoes has not yet been established, even though intensive control operations have been carried out for several years in many areas, but we have no assurance that insecticide-resistant strains of *Anopheles* cannot develop.

It is my opnion that intensive and widespread application of DDT and other insecticides to both larval and adult culicine mosquitoes is the primary reason for the appearance of resistant strains in certain areas. Antilarval measures other than the use of insecticides and the application of antiadult measures to minimum size localized areas may prevent or delay the appearance of insecticide-resistant mosquitoes. Further research to develop new mosquito insecticides and an expanded program of basic studies on the resistance problem are needed.

REFERENCES

Bohart, R. M., and W. D. Murray 1950, DDT resistance in *Aedes nigromaculis* larvae, *Calif. Mosquito Control Assoc. Ann. Conf., Proc. and Papers* **18**: 20–21.

Bruce, W. N., and G. C. Decker 1950, Housefly tolerance for insecticides, *Soap and Sanit. Chem.* **26**(3): 112–125, 145–147.

Deonier, C. C., T. L. Cain, Jr., and W. C. McDuffie 1950, Aerial spray tests on adult salt-marsh mosquitoes resistant to DDT, *Jour. Econ. Ent.* **42**: 506–510.

Deonier, C. C., and I. H. Gilbert 1950, Resistance of salt-marsh mosquitoes to DDT and other insecticides, *Mosquito News* **10**: 138–143.

Fay, Richard W., W. C. Baker, and Mary M. Grainer 1949, Laboratory studies on the resistance of *Anopheles quadrimaculatus* (Say) to DDT and other insecticides, *Jour. Natl. Malaria Soc.* **8**: 137–146.

Gahan, James B., Wilbur G. Downs, and Heliodoro Celis 1949, Control of *Anopheles pseudopunctipennis* in Mexico with DDT residual sprays applied in buildings. Part II. *Amer. Jour. Hygiene* **49**: 285–289.

Gjullin, C. M., and R. F. Peters 1952, The present status of mosquito resistance to insecticides in California, *Mosquito News* (in press).

King, W. V. 1950, DDT-resistant house flies and mosquitoes, *Jour. Econ. Ent.* **43**: 527–532.

Mosna, E. 1947, Su una caratteristica biologica del *Culex pipiens autogenicus* di Latina, *Riv. di Parassitol.* **8**: 125–126.

Mosna, E. 1948, *Culex pipiens autogenicus* DDT -resistenti e loro controllo con Octa-Klor e esaclorocicloesano, *Riv. di Parassitol.* **9**: 19–25.

Sumerford, W. T., M. B. Goeffe, K. D. Quarterman, and S. L. Schenck 1951, The potentiation of DDT against resistant houseflies by several structurally related compounds, *Science* **114**: 6–7.

Trapido, Harold 1951, The toxicity of DDT to *Anopheles claviger* (Meig.) in Sardinia and on the Italian mainland, *Jour. Natl. Malaria Soc.* **10**: 266–271.

HOUSE FLY RESISTANCE TO CHEMICALS[1]

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It is no doubt significant that housefly resistance to DDT was first recognized and reported in Europe, where DDT came into commercial use in 1944 and was used extensively by civil and military personnel in 1945. In 1947, Sacca and Missiroli attributed the failure of DDT to control flies in Italy to the presence of a variety of house fly which had become resistant to DDT. About the same time Wiesmann (1947) reported house fly resistance in Sweden. Two years later Keiding and Van Deurs (1949) in three short paragraphs summarized the course of events which was apparently common throughout Europe, as follows:

"Already in 1946 there were some isolated complaints of failing effect, and in the summer of 1947 the fly control failed in a great many places. It was attributed to careless spraying, as well as to the unusually hot summer, the effect of the high temperature being an increased production of flies in the dung-heaps, etc., a decrease in the toxicity of DDT to insects, and possibly a more rapid deterioration of the deposits on limewashed surfaces.

"Early in 1948 we received accounts from various places of flies which 'could not be killed with DDT,' even with much higher dosages than those usually recommended. Houseflies (adults, pupae and larvae) were collected from six of these places and the strains so obtained (r_1, r_2, r_3, etc.) were bred by a modified N.A.I.D.M. procedure in parallel with the non-resistant laboratory strain (lab.) which has been bred from flies taken on a farm near Copenhagen in October 1945.

"The results of the first laboratory tests showed unmistakably that all six *r*-strains were several times more resistant to DDT than the laboratory strain."

Except for the fact that each event came to pass about one year later, the account of Keiding and Van Deurs might be taken almost word for word as the historic record of the development of house fly resistance to DDT in the United States. Here reports that DDT was not performing as well as it had in the past were quite prevalent in 1947. By 1948 an alarming degree of DDT resistance was reported in California by March and Metcalf (1949), and similar conditions were reported by numerous other workers at scattered locations throughout southern, eastern, and central United States.

In Illinois, where DDT came into fairly general use in 1945, the development of house fly resistance to DDT followed the same pattern reported elsewhere, but it is possible that here we have a more complete historic record of events. Each year flies collected from the field were cultured in the laboratory and their LD 50's were determined. In the years 1945 to 1948, all of the field samples collected were from farms where DDT was being used rather extensively in fly control. In 1949 and succeeding years, however, field samples were collected at random and in theory, at least, represented all methods and perhaps all degrees of chemical usage. As shown in Table 1, resistance developed slowly at first but became a significant factor in fly control by 1948. In 1949, 86 of the 94 samples collected at random on widely scattered farms in Illinois showed a significant degree of resistance. In 1950, all 36 of the samples collected throughout the state showed a moderate to high degree of DDT resistance. This year (1951) 81 field samples were collected and tested for DDT resistance or tolerance. All were found to be resistant to DDT and it is worthy of note that even with a decline in DDT usage the magnitude of resistance increased about five fold.

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With the appearance of DDT resistance, farmers began switching to other insecticides and as a result, 8 per cent of the 36 samples collected in 1950 showed a significant degree of resistance to lindane, chlordan, and dieldrin. In the 1951 survey (Table 2) 100 per cent of the samples showed resistance to DDT, 95 per cent showed resistance to methoxychlor, and approximately 50 per cent showed resistance to lindane and dieldrin. It was also apparent that in most cases the amount of each chemical required to kill the resistant flies encountered in the field was many times that required to kill normal or susceptible flies.

TABLE 1

DDT resistance observed in field-collected strains of house flies (1945–1951)

YEAR	NO. OF FARMS TESTED	DDT, LD-50		PER CENT RESISTANT
		Range	Average	
1945	3	0.18–0.2	0.19	?
1946	3	0.30–0.45	0.34	?
1947	4	0.7 –0.82	0.75	?
1948	8	3.6 –9.3	8.5	?
1949	94	1.1 –15.7	5.1	87
1950	36	3.3 –42.0	9.9	100
1951	81	9.0 –125.0	45.7	100

It appears that we have now reached a point where DDT and related materialss such as methoxychlor, are of little or no practical value in fly control in Illinois, and with approximately 50 per cent of our farms producing flies that are resistant to lindane and dieldrin, there is reason to believe that most if not all of the chlorinated hydrocarbon insecticides will have lost their usefulness within a year or two. We doubt if Illinois is exceptional in this regard; at any rate, we are led to believe that in some other sections of the country where chlorinated hydrocarbons have been used more extensively and where house flies produce more generations each year, conditions may be even worse.

So much for the predicament we find ourselves in today with regard to fly control. Now let us turn to a few of the elementary things we have learned about house fly resistance to chemicals.

In a previous paper the writers, Bruce and Decker (1950), have shown that the initial processes of segregation and selection leading up to the establishment of high levels of resistance proceed slowly at first, but that when resistance is once established its intensification is rapid and proceeds to equilibrium with the toxicant found in the strain's environment.

Additional studies have confirmed the original findings and have shown that the course followed in the development of resistance is about the same for all of the chlorinated hydrocarbon insecticides studied. Usually continuous chemical pressure during six to twenty generations is required to develop a significant degree of resistance (5 to 10 times normal). After that, resistance may double, triple, or quadruple with each succeeding generation, reaching a level 100 to 1,000 times normal in 6 to 20 additional generations (Figure 1).

There is an abundance of evidence tending to show that the acquisition of tolerance for one chemical may predispose a strain to the rapid development of resistance to some other chemical. For example, when a strain of flies (DDT I) resistant to DDT is subjected to treatment by another insecticide such as methoxychlor, lindane, or dieldrin, resistance to the new chemical is developed rapidly. The period of 5 to 10 generations otherwise required to complete the process of segregation and selection is apparently eliminated and the processes of intensification begin at once (Figure 2). Thus, as a comparison of Figures 1 and 2 will disclose, a very high degree of resistance is attained in about half the time required to attain the same degree of resistance starting with a normal or susceptible strain.

TABLE 2

Resistance to insecticides observed in 81 farm-collected strains of house flies in 1951, expressed in micrograms per fly

INSECTICIDE	% OF STRAIN SHOWING RESISTANCE	RANGE OF LD-50'S	AVERAGE LD-50	LD-50 OF INSECTICIDES TO NORMAL FLIES
DDT	100.0	9.0 -125.0+	45.7	0.33
Methoxychlor	95.0	1.5 -125.0+	36.6	0.99
Lindane	49.4	0.07-13.0	2.54	0.03
Dieldrin	49.4	0.04-41.0	8.76	0.02

In general, the treatment of adult flies alone does not result in the rapid development of a high degree of fly resistance to or tolerance for DDT or any of the other insecticides. On the other hand, the treatment of the larval breeding media, alone or in addition to adult exposure, greatly intensifies natural selection and inbreeding, thereby hastening the development of resistance. Obviously, to produce a resistant strain of flies in the laboratory or in nature, the chemical exposure must not be completely lethal, otherwise there will be no survivors and the population would be eliminated. When cultures which had 80 p.p.m. of DDT added to the N.A.I.D.M. larval media were compared with cultures where adults were exposed in treated cages only, no significant difference in tolerance was noted in the first three generations. However, by the sixth generation the flies from cultures in which the breeding media was treated had developed a degree of resistance five to ten times that of normal flies (Table 3). In ten generations the resistance in these strains was over 300 times normal, whereas the flies from cultures receiving adult exposure only still showed only nominal resistance, four to five times normal.

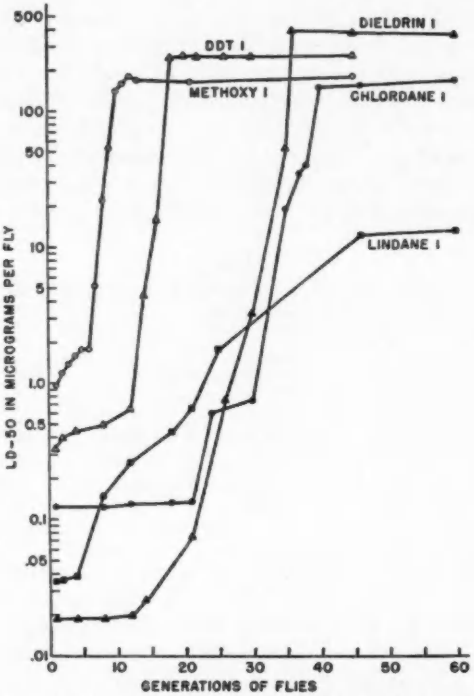

FIG. 1. LD-50 curves for five strains of flies obtained by topical application of chemical on female house flies for successive generations during the development of resistance. Larvae and adults of each generation exposed to the respective chemicals.

There is evidence that the contamination of breeding media likewise contributed to the development of DDT resistance under conditions prevailing in the field. DDT resistance appeared first and was intensified most rapidly on farms where sanitation was not too good and where spraying operations resulted in serious contamination of the manure and similar breeding media. The average insecticide application on a ceiling area will usually result in a portion of the toxicant finding its way to the floor through drift and dripping. The floor often appears as wet as the ceiling. In order to determine to what extent the litter in sprayed buildings was contaminated, samples were taken from the floors of 12 buildings and analyzed for DDT. The DDT content of the straw-manure-debris mixtures ranged from 215 to 448 p.p.m.

The frequent observance of evident symptoms of insecticide poisoning and a high rate of mortality soon after emergence among adult flies reared from field-collected pupae have indicated the possible storage of the toxicant in the larval stage. In one instance these effects were evident for more than three months after a single residual application of a 0.3 per cent dieldrin spray.

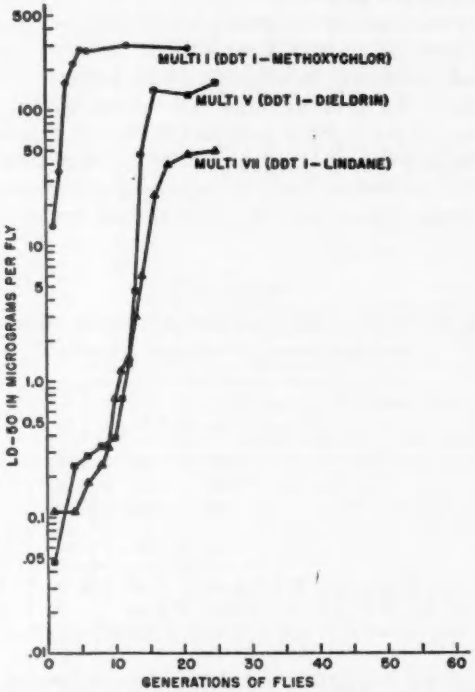

Fig. 2. LD-50 curves for three strains of flies produced from the DDT I-resistant strain by exposing adults and larvae of each generation to the chemicals indicated. (LD-50 values obtained by topical application to adult female flies.)

Time and space will not permit a detailed discussion of many resistant strains, but perhaps a resume of the resistance characteristics of a few strains typical of the thirty maintained in the Urbana laboratories will serve to illustrate several points. The 24-hour LD-50 values for several insecticides (in micrograms per fly) when applied topically to several resistant strains developed in the laboratory are given in Table 4. As previously indicated, the acquisition of tolerance for one chemical may predispose a strain to resistance for other chemicals. By reading horizontally across the line for each strain and comparing the LD-50 value for each chemical with the normal value given in line 1, we can see to what extent the strain's response to any given chemical has changed in the course of developing a high degree of tolerance for another.

It appears that flies exposed to synergized pyrethrins and para-oxon develop resistance very slowly and that, even after 30 to 40 generations of inbreeding, the order of tolerance attained for these chemicals is only 10 to 20 times that of normal flies. Furthermore, it is apparent that the acquisition of a high degree of tolerance for the other chemicals tested had little effect on the flies' response to synergized pyrethrins and para-oxon.

All of the other materials tested produce a high degree of resistance in a comparatively short time and all tend in some degree to induce a certain degree of resistance to other compounds. In acquiring a high degree of resistance to DDT, flies also acquire a fair degree of resistance to the closely related compound methoxychlor, but only a slight degree of resistance to lindane, chlordan, and dieldrin. Chlordan, dieldrin, and lindane apparently share a close relationship, and resistance to any one of the three carries with it a fair degree of resistance to the other two. The relationship between chlordan and dieldrin seems to be particularly close.

TABLE 3

LD-50's for DDT applied topically to adults of successive generations of flies

Results expressed in micrograms per fly

EXTENT OF TREATMENT	GENERATION				
	F_1	F_3	F_6	F_8	F_{10}
Cage—adults	0.33	0.34	0.38	1.22	1.65
Media—larva	0.33	0.41	3.30	14.4	>100.00
Media and cage	0.32	0.36	2.80	21.6	>100.00
	F_1	F_3	F_6	F_9	F_{12}
Cage—adults	0.33	0.43	0.66	0.83	1.20
Media and cage	0.31	0.49	1.65	72.0	>100.00

When a strain of flies resistant to one chemical was subjected to treatment by another chemical, resistance to the second toxicant was acquired rapidly and here again the inter-chemical relationships noted above tended to prevail. Starting with the Multi I strain, which was resistant to DDT and methoxychlor, the application of a mixture of insecticides, "Shot Gun Treatment," produced the maximum observed resistance to all of the materials simultaneously.

It seems fully possible, if not indeed probable, that tolerance for insecticides may be extremely variable and induced by the independent or joint action of many independent characters. It has been demonstrated (Sternburg *et al.* 1950) that in some cases resistance is due to the fly's ability almost immediately to metabolize or at least decompose DDT into harmless components. At the same time, there are some highly resistant strains in which the DDT is not immediately decomposed and in which sizeable amounts of DDT can be found in the blood and body tissues of the living insects. In still other strains it appears that resistance or tolerance may be due largely to varied morphological characters or

TABLE 4

Twenty-four hour LD-50's for several insecticides (in micrograms per fly) when applied topically to the several resistant strains of house flies developed in the laboratory

STRAINS	GENERATIONS EXPOSURE OF ADULTS AND LARVAE TO THE INSECTICIDE TO REACH A MAXIMUM LEVEL OF RESISTANCE		ORIGIN OF STRAIN	INSECTICIDE						
	Generations	Insecticide		DDT	Methoxychlor	Lindane	Chlordan	Dieldrin	10:1 mixture of piperonyl-butoxide and pyrethrins	Paraoxon
Laboratory susceptible			N.A.I.D.M.	0.33	0.99	0.03	0.12	0.019	1.13	0.045
Pyro I	30	Pyrenone	lab.	0.68	1.10	0.16	0.32	0.026	>23.20	0.07
Para-oxon	40+	Para-oxon	lab.	0.45	1.00	0.05	0.15	0.02	1.22	>0.51
DDT I	18	DDT	lab.	260.5	14.40	0.11	0.24	0.048	1.77	0.07
DDT III	?	DDT	field	26.1	8.2	0.04	0.09	0.016	1.16	—
DDT IV	9	DDT	lab.	195.0	8.7	0.04	0.29	0.04	1.30	0.05
Lindane	46	Lindane	lab.	1.20	1.10	12.7	1.86	0.40	1.40	0.05
Dieldrin	36	Dieldrin	lab.	2.50	3.90	0.60	101.0	390.0	1.90	0.07
Chlordan	40	Chlordan	lab.	0.72	1.5	0.65	150.2	7.25	1.22	0.07
Multi I	4	Methoxychlor	DDT I	298.0	220.0	0.12	0.23	0.08	1.80	0.07
Multi VI	19	Lindane	Methoxychlor	0.80	>100.0	40.6	2.70	0.70	1.40	0.06
Multi VII	17	Lindane	DDT I	275.0	16.70	48.3	0.96	0.60	1.50	0.09
Multi IX	19	Mixture*	Multi I	325.0	225.0	148.4	131.3	378.9	2.00	0.07

* A mixture of chlordan, lindane, dieldrin, toxaphene, DDT, methoxychlor.

variations in habit rather than to chemical factors. We may therefore reasonably expect that strains of different origin produced under quite distinct environmental conditions will vary distinctly in their response to varied laboratory conditions.

In an attempt to reconcile some differences of opinion, four laboratories located in various parts of the country exchanged resistant cultures and proceeded with testing in accord with the technique normally used in the respective laboratories. The results obtained were in general agreement and the discrepancies encountered could be accounted for by considering the variation in the techniques employed.

TABLE 5

The range of sampling error where three masses of 1500 eggs each (not mixed or randomized) were taken from cultures of five DDT-resistant strains of flies

LD-50 values in micrograms per fly

STRAIN	SAMPLE		
	1	2	3
A	400	510	250
B	5	10	4
C	340	400	525
D	86	172	200
E	15	19	22

TABLE 6

Per cent mortality of F_1 generation flies for three sub-cultures developed from a single DDT-resistant strain by selecting adults on the basis of early and late emergence

SELECTION	MICROGRAMS OF DDT PER FLY		
	0.5	5.0	50.0
Early emergence	8	24	96
Late emergence	0	0	76
Random mixed	0	8	92

Some rather large discrepancies were obviously due to sampling errors or the effect of adverse environmental conditions encountered in transit. In several cases the shipment of a duplicate sample corrected the error. In pursuing the sampling error problem, the writers found that if the eggs were not thoroughly mixed before sampling, and individual groups or masses of eggs were selected, the tolerance for DDT of the cultures produced might vary as much as 50 per cent (Table 5). It was also found that cultures developed from the first eggs produced showed much less resistance to DDT than did cultures developed from eggs deposited by flies emerging one or two days later.

In cultures having some degree of heterogeneity, the more susceptible flies emerge first and this fact may account for some of the difference of opinion that has developed regarding the stability or permanency of resistance. The writers have consistently maintained that if no outbreeding were permitted, resistant strains of flies would, in the complete absence of further exposure to the chemical, retain their resistance at a constant level for many generations. Insofar as genetics is concerned, this concept is apparently valid. Under laboratory conditions, however, it would be theoretically possible to demonstrate either rapid reversion or intensification by merely selecting the earliest or latest emerging flies to perpetuate the colony. To test the validity of this hypothesis, the writers selected a field-collected strain showing 60 times normal DDT resistance, which had been held in the laboratory for one year at a rather constant LD-50 value of 20 micrograms per fly. From it three sub-cultures were developed. For one sample the earliest-emerging flies were selected, for the second the late-emerging flies were selected, and for the third, random selection was employed to maintain the status of the original culture. In the F_3 generation, Table 6, we found an apparent three-fold reduction in the number of resistant flies in the culture developed from the early-emerging flies, and a three-fold reduction in the number of susceptible flies in the culture developed from the late-emerging flies.

Research on house fly resistance to chemicals is under way at many institutions, and while the genetic aspects of the problem and the exact nature of resistance have not as yet been clearly defined, much progress is being made. Unfortunately, some investigators have tended to make some point of the variance between their results and the results obtained by others without giving adequate consideration to the fact that the genetic complex of the strains under consideration might be quite different or that variables in the techniques followed might easily produce the variations observed.

There is reason to hope that a better understanding of the variables mentioned and others encountered in resistance investigations will eliminate much of the apparent confusion which is now in some degree retarding the progress of research on house fly resistance to chemicals.

BIBLIOGRAPHY

Bruce, W. N. and G. C. Decker 1950, House fly tolerance for insecticides, *Soap and Sanit. Chem.* **26**: 122–125, 145–147.

Keiding, J. and H. Van Deurs 1949, DDT resistance in houseflies in Denmark, *Nature* (London) **163**: 964–965.

March, Ralph B. and R. L. Metcalf 1949, Laboratory and field studies of DDT resistant houseflies in southern California, *The Bulletin*, Dept. of Agr., State of California **38**: 93–101.

Missiroli, A. 1947, Riduzione o eradicazione degli anofeli, *Riv. di Parassitol.* **8**: 141–169.

Sacca, Giuseppe 1947, Sull'esistenza di mosche domestiche resistenti al DDT, *Riv. d. Parassistologia* **8**: 127–8.

Sternburg, James, C. W. Kearns, and W. N. Bruce 1950, Absorption and metabolism of DDT by resistant and susceptible houseflies, *Jour. Econ. Ent.* **43**: 214–219.

Wiesmann, R. von 1947, Untersuchungen uber das physiologische Verhalten von *Musca domestica* L. verschiedener Provenienzen, *Schweiz. Ent. Gesell. Mitt.* **20**: 484–504.

THE CURRENT STATUS OF PHYSIOLOGICAL STUDIES ON DDT RESISTANCE

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INTRODUCTION

Insecticide resistance, a development first noticed some fifty years ago (Smith, 1897), is a major problem confronting the medical and economic entomologist today. Realization of this unpleasant fact is growing rapidly (Babers, 1949, 1951), thanks mainly to the conspicuous failure of DDT to provide satisfactory control of flies in some areas where earlier this agent was highly effective. Disconcerting as this situation is, still more so is the experience that empirical attempts at solution of the difficulty have served to emphasize the gravity of the present state of affairs rather than to provide the practical answers sought. Attention has thus been directed anew toward the need for a better understanding of the basic phenomena involved. It is the purpose of this discussion to present a brief evaluation of the physiological data which are now available for application to the problem. Since most of the pertinent work has dealt with DDT, and since space is limited, attention will be focussed on this compound; but it should be stressed that what has occurred in the field with DDT and houseflies is merely symptomatic of what may be expected, and in some cases is already being encountered, with a variety of other residual insecticides and insect species. Although, fortunately, such problems are only beginning to assume practical significance in the field of malaria control, the menace of such developments in this area is so great that every malariologist will wish to be armed against them if and when they should arise. A similar attitude toward other insect borne diseases is equally justified by current experience in the field.

A remedial approach on a physiological basis would appear ideally to comprise the following steps: (1) discovery and complete definition of the significant mechanisms of intoxication, a process which ordinarily requires extensive preliminary investigation of the physiology of normal insects; (2) development of equivalent data in regard to the mechanisms whereby a resistant population evades the usual effects of the toxicant; (3) synthesis of the resulting information into the design of control measures which will not only be immediately effective but which will prevent a recurrence of the problem. Let us consider under these headings what is known in regard to DDT.

MECHANISM OF INTOXICATION

The now familiar symptoms of DDT poisoning in insects early suggested the nervous system as the site of action. Within this locus, Läuger and colleagues (1944) argued that the primary site is peripheral. Their hypothesis has gained precision through the work of other laboratories (Yeager *et al.*, 1945; Bodenstein, 1946; Roeder *et al.*, 1946; Tobias *et al.*, 1946; Welsh *et al.*, 1947) and we now know that DDT in reasonably low concentrations makes some sensory cells and nerve fibers abnormally excitable, so that they respond with multiple discharges after single stimuli, or even in the absence of a recognizable stimulus. Identical effects have been observed after treatment with a few structurally related compounds, but do not as a rule follow the application of other types of insecticides, even though many of these are obviously neurotoxins also. Evidence for the involvement of the *central* nervous elements in DDT poisoning is conflicting. Hyperactivity is seen here too (e.g., Tobias *et al.*, 1946) but in most experiments the probability has not been ruled out that it is a secondary result of the augmented flow of incoming signals.

Similarly, the striking increase in muscular activity, which up to a point becomes more and more violent and less coordinated as poisoning progresses, is interpreted as a reflex result of the increase in nervous discharge, since it is reduced or abolished by nerve blocking agents (Yeager *et al.*, 1945; Bodenstein, 1946), while no direct effect of DDT on the activity of muscle tissue has been found. Most or all of the accompanying respiratory increase appears likewise to be secondary, as result of the extra muscular work; however, discrepancies reported in the time course of the two phenomena (Lung, 1945; Buck *et al.*, 1949) leave a suspicion that there may also be some more direct effect on respiratory metabolism.

How DDT produces the observed instability of peripheral nerve is still a mystery. Gordon and Welsh (1948), with an eye to the affinity of DDT for lipoids, postulated a displacement of bivalent cations from the nerve membrane leading to depolarization; and in some experiments with *Crustacea* were able to demonstrate a restorative effect of added calcium. Such observations are very suggestive, but by no means clinching; and in any case it is not clear that nervous instability is the actual cause of death.

To account for the latter, the theory has been proposed that the abnormal activity burns out the supply of essential foodstuffs. However, efforts to repair the supposed deficiency by injecting or feeding energy-rich substrates have failed to increase the survival of poisoned insects (Merrill *et al.*, 1946). This is true also of administration of anticonvulsants or anesthetics, which may suppress outward symptoms and utilization of energy without altering the time at which death ensues. Moreover, starved specimens have survived much greater depletion of carbohydrate and water than has been measured in DDT poisoned insects at the time of death.

Alternatively it has been suggested that death is due to an accumulation of toxic metabolites, and increases in lactic acid and in ketone bodies have been measured (Ludwig, 1946; Merrill *et al.*, 1946). But is has not been shown that such phenomena are the cause rather than the result of initial hyperactivity followed by a general breakdown of physiological function; and in roaches the observed ketosis in the terminal stages of DDT poisoning was less by a factor of 1000 than the dose of injected acetone required to produce a toxic effect.

Studies of the possible action of DDT on a variety of enzyme systems have generally yielded negative or inconclusive results (Ludwig, 1946; Barron, 1945;

Richards *et al.*, 1945; Tobias *et al.*, 1946). An exception is cytochrome oxidase, an enzyme concerned in the final steps of oxidative metabolism. Partial inhibition of this catalyst by DDT has been demonstrated in vitro and in poisoned flies (Sacktor, 1949; Johnston, 1951), yet proof is still lacking that such inhibition is a significant factor in the mechanism of intoxication. In fact, a similar degree of inhibition has been produced in vitro by DDE (Johnston, 1951), which has very little toxicity for insects, and by the *o-p′* and *o-o′* isomers of DDT (Sacktor, 1951). Slight inhibition of phosphatidases and alkaline phosphatase by DDT has also been reported (Läuger *et al.*, 1944; Monche, 1950)

It appears then that while we can speak with some assurance about a number of things which DDT does *not* do, we can say relatively little that is definite about what it does and how. Our continuing ignorance of the mechanism of poisoning is an obstacle to solution of the resistance problem which every effort should be made to overcome. To summarize the positive information: we know that DDT in physiological concentrations causes characteristic symptoms in certain parts of the nervous system; and that it can act as an inhibitor of one or two vital enzymes. But what is the finer mechanism in each case? Are the two processes related, and if so, how? Is either, or are both, responsible, directly or indirectly, for the lethal effect? Or are the truly significant mechanisms yet to be discovered?

MECHANISM OF RESISTANCE

With this meager background on the mechanism of intoxication, the problems of the physiology of DDT resistance must be approached. But first a word about genetics, although this aspect of insecticide resistance has been even less studied. Resistance to adversity, whether the latter be inherent in nature or the result of man's intervention, must be a characteristic of organisms that are to survive. We need not be surprised then that insects, with their long history of successful evolution, can muster the ability to thwart our best efforts at rearranging the conditions of life to our own advantage. Contact with strange chemicals is but one of many factors which make life hard for insects, and, in general, the known principles of population genetics, derived from other situations, seem adequate to account for the facts which have been recorded in studies of resistance to insecticides. The individuals of every population vary in susceptibility to any given toxicant, and this variation is dependent, in part at least, upon hereditary factors. Hence, every population has potential resistance in some degree; but since the initially predominant genotypes are those best adapted to the normal environment, one presumes that genes responsible for exceptional tolerance toward a new chemical will ordinarily be recessives present in low frequency. Introduction of the chemical at once alters selection pressure in favor of these genes and of any subsequent mutations which contribute to survival in the presence of the toxicant. If the new selection pressure exerted by the poison is high, and if homozygosity with respect to the genes for resistance is compatible with survival and reproduction, a new, resistant set of genotypes carrying these factors will replace the old in relatively few generations. Once homozygosity has been attained throughout the population, resistance will be maximal and fixed; and will continue unabated, except for the possibility of mutations in the reverse direction, even in the absence of any further exposure to the agent.

Numerous differences have been reported for the permanence of resistance in various strains of flies and other insects (cf. Babers *et al.*, 1951). It is likely that many of these variations depend on differences in the degree of homozygosity for resistance; and there are also indications, in some but not all experiments, that a high degree of resistance may be linked with low viability, slower development, decreased reproductive potential, or harmful abnormalities of structure (Bruce, 1949; Primentel *et al.*, 1950; Snyder, 1951; Fay et *al.*, 1949). Where this is so, there will evidently be pressure ***against*** the development of homozygosity for resistance despite the survival value of resistant traits. The capacity of such strains for the development of resistance is limited, though it may still be great enough to pose a serious problem in practical control; and chances are that these populations will revert rather rapidly toward normal sensitivity after exposure has ceased. Unfortunately, not all populations share these shortcomings. Exact information about the genetics of insecticide resistance could obviously be of great assistance in the design of appropriate control measures. It is to be hoped therefore that research will be undertaken which will provide a foundation of fact in lieu of the largely hypothetical considerations outlined in the preceding paragraphs.

The physiological nature of DDT resistance is still highly uncertain. While it has been claimed by some that slight structural differences, such as thickness of tarsal cuticle, exist between certain susceptible and DDT resistant strains of flies (Wiesman, 1947), other studies have failed to provide corroboration (March *et al.*, 1950). Factors of this sort are probably relatively unimportant in the mechanism of resistance, especially since the latter has been found independent of the route of administration (Bettini, 1948; March *et al.*, 1949). A decreased rate of penetration of the poison may contribute to resistance in the field, but evidently cannot be solely responsible. Other theoretically possible mechanisms are numerous. Behavior may shift to patterns which minimize the likelihood of accumulating a lethal dose (King *et al.*, 1949; Bruce, 1949; Bruce *et al.*, 1950). Or, assuming that the toxicant enters and blocks or alters some essential function, the resistant organism may possess a bypass. Alternatively the agent may be offered some non-vital site with which to combine preferentially, thus providing a physiological barrier which impedes access of the poison to systems necessary for life. Again, the toxic material may be excreted, or destroyed in the body, at a more rapid rate than normal. Actually it is probable that a combination of several of these or other mechanisms is responsible for the resistance encountered in field operations, a fact which increases the difficulty of physiological analysis as well as the burden of control.

Though only a few investigators have as yet accepted the challenge of this complex problem, probing into the last of the possible mechanisms mentioned above is at present yielding fruitful results with DDT resistant insects. Significant correlations have been demonstrated between resistance or survival and the conversion of DDT to the non-toxic derivative, DDE (Perry *et al.*, 1950; Sternburg *et al.*, 1950, 1950a; March *et al.*, 1950; Lindquist *et al.*, 1951). The enzymatic nature of this reaction in vivo is indicated by its absence from heated tissue (Hoskins, 1951), although the system responsible has neither been isolated nor identified. Other studies have shown that some synergists or activators, which enhance the toxicity of DDT to resistant flies, reduce the ability of the insects to perform the conversion to DDE (Perry *et al.*, 1950); and that mortality is then proportional to the degree to which conversion is suppressed (Perry, 1951). Several of the known activators, though by no means all, are in one respect or another similar chemically to DDT (Sumerford *et al.*, 1951; Metcalf *et al.*, 1951); it is theorized that they may therefore readily replace DDT as substrate for the detoxifying enzyme, thus leaving the insecticide free to exert its lethal effect.

These studies present a more coherent and encouraging picture than much of the other physiological data in regard to DDT, yet the detoxification theory, like other current theories concerning the mechanisms of DDT poisoning and resistance, is not without its discrepancies. The measured rate of conversion often seems too slow; and resistant flies, in spite of having changed much DDT to DDE, may still contain enough of the toxicant to kill several susceptible specimens and yet show no signs of poisoning (Winteringham *et al.*, 1951). What prevents this unchanged DDT from acting? Evidently not the mere presence of DDE, since resistant flies of some species, reported to be full of DDE, may develop all the usual symptoms if cooled after treatment; while pretreatment of susceptible flies with DDE fails to confer resistance upon them. Also difficult to reconcile with the detoxification theory is the fact that the rate of conversion, at the low dosage levels where comparison is possible, is no different in some susceptible and resistant strains (Perry, 1951), which suggests that resistant flies may be converting more DDT to DDE simply because they survive long enough to do so. From this viewpoint, increased detoxification would be a result of resistance, rather than its cause. Though some of these objections to the theory seem serious at present, they are not necessarily insurmountable. Further investigation must be relied upon to decide the issue.

A somewhat different line of attack has been opened by observations (1) that adults of a resistant strain have more cytochrome oxidase and (2) that pupae of the same line, with less of this enzyme than the susceptible strain, perform an abnormally high percentage of their respiration via an alternative system (Sacktor, 1950, 1951). If the significance of inhibition of cytochrome oxidase in DDT poisoning had been established, one could see that these resistant flies would be in a better position to withstand a partial blocking of this vital metabolic pathway, the exact mechanism being different in the two stages studied. Proof of the premise is still wanting, but, being already in the realm of hypothesis, one may as well go on to suggest a possible connection between this supposed mechanism and that of detoxification. Conversion of DDT to DDE, outside the body, is known to be promoted by various factors, among them the presence of metallic ions, especially iron and aluminum (Fleck *et al.*, 1944). This last observation, as well as the fact that HCl is liberated, raises a strong suspicion that oxidation-reduction reactions may be important in the series of changes involved in the conversion; if so, the detoxification process might well be interwoven *in vivo* with biological oxidation-reduction systems such as that of which cytochrome oxidase is a part.

APPLICATION OF PHYSIOLOGICAL DATA TO PRACTICAL CONTROL OF RESISTANT INSECTS

From the data sketched above it is abundantly evident that though physiological studies have given rise to much intriguing speculation, they have not yet progressed to the point where they furnish a firm basis for decisive recommendations as to how to deal practically with the problems of insecticide resistance. This is not a criticism of our colleagues who have been engaged in the work I have tried to summarize, for they have been few in number and on the whole have received little encouragement and a minimum of support. Under the circumstances they deserve congratulation for the amount of light they have already shed on a highly complex and difficult problem, for having invented and perfected delicate techniques which will be useful elsewhere also, and for having developed numerous leads which it will obviously be profitable to pursue further. As an illustration of what we may hope to realize when such efforts are carried to their logical conclusion, it is apparent that if DDT resistance in insects can be linked definitely to specific enzymatic detoxification systems, the latter identified and their properties ascertained, we shall then be in a position to recommend the inclusion of specific inhibitors or blocking agents in the screening program for DDT activators, and to suggest to the synthetic chemist what types of new compounds may be expected to yield the desired effect. One might even dream of presenting the detoxification mechanism with a preferred substrate which would produce DDT or some other toxic compound on conversion!

Obviously there are great deficiencies of knowledge to be made good before any such ideal way around our present troubles can be cleared, and until something of the sort has been accomplished we are forced, so far as chemical control is concerned, to rely on the empirical approach. Of necessity this consists simply of throwing one new insecticide after another into the gap reopened all too rapidly by the development of resistance to the last, so that the procedure, though inevitable at present, cannot be regarded as a promising or economical road to a permanent solution. Only thorough comprehension of all the mechanisms concerned in the action of insecticides and in resistance to them will afford us the basis for a truly intelligent and efficiently directed program. In view of these facts and the impact of the resistance problem on human health and welfare, a substantial increase in emphasis on the basic aspects of research directed toward insect control is not only justified but imperative.

REFERENCES

1. **Babers**, F. H. 1949, Development of insect resistance to insecticides, U. S. Dept. Agric. Mimeographed report No. E-776.
2. **Babers**, F. H. **and** J. J. **Pratt, Jr.** 1951, Development of insect resistance to insecticides II, U. S. Dept. Agric. Mimeographed report No. E-818.
3. **Barron**, E. S. G. 1945, (personal communication).

4. Bettini, S. 1948, Contributo allo studio della resistenza all' azione del DDT nelle mosche domestiche, *Riv. di Parassitol.* **9:** 137–142.
5. Bodenstein, D. 1946, Investigation on the locus of action of DDT in flies (Drosophila) *Biol. Bull.* **90:** 148–157.
6. Bruce, W. N. 1949, Latest report on fly control, *Pest Control* **17:** 7, 28.
7. Bruce, W. N. and G. C. Decker 1950, House fly tolerance for insecticides, *Soap and Sanit. Chem.* **26:** 122–125, 145–147.
8. Buck, J. B. and M. L. Keister 1949, Respiration and water loss in the adult blowfly, *Phormia regina*, and their relation to the physiological action of DDT, *Biol. Bull.* **97:** 64–81.
9. Fay, R. W., W. C. Baker and M. M. Grainger 1949, Laboratory studies on the resistance of *Anopheles quadrimaculatus* to DDT and other insecticides, *J. Nat. Malaria Soc.* **8:** 137–146.
10. Fleck, E. E. and H. L. Haller 1944, Catalytic removal of HCl from some substituted α-trichloroethanes, *J. Am. Chem. Soc.* **66:** 2095.
11. Gordon, H. T. and J. H. Welsh 1948, The role of ions in axon surface reactions to toxic organic compounds, *J. Cell. Comp. Physiol.* **31:** 395–420.
12. Hoskins, W. M. 1951, As reported in *Chem. and Engin. News* **29:** 3969–3970.
13. Johnston, C. D. 1951, The in vitro effect of DDT and related compounds on the succinoxidase system of rat heart, *Arch. Biochem. and Biophys.* **31:** 375–382.
14. King, W. V. and J. B. Gahan 1949, Failure of DDT to control houseflies, *J. Econ. Ent.* **42:** 405–409.
15. Laug, E. P. 1945, Report to Insect Control Committee, *OSRD*; April, 1945.
16. Läuger, P., H. Martin and P. Müller 1944, The constitution and toxic effect of botanicals and new synthetic insecticides, *Helv. Chim. Acta* **27:** 892–928.
17. Lindquist, A. W., A. R. Roth, W. W. Yates, R. A. Hoffman and J. S. Butts 1951, Use of radioactive tracers in studies of penetration and metabolism of DDT in house flies, *J. Econ. Ent.* **44:** 167–172.
18. Ludwig, D. 1946, The effect of DDT on the metabolism of the Japanese beetle, *Popilia japanica* Newman, *Ann. Ent. Soc. America* **39:** 496–509.
19. March, R. B. and R. L. Metcalf 1949, Laboratory and field studies of DDT resistant houseflies in southern California, *Calif. State Dept. Agric. Bull.* **37:** 93–101.
20. March, R. B. and L. L. Lewallen 1950, A comparison of DDT-resistant and non-resistant house flies, *J. Econ. Ent.* **43:** 721–722.
21. March, R. B. and R. L. Metcalf 1950 a, Investigations of insecticide-resistant houseflies in southern California, *Calif. Mosquito Control Assoc. Conf. Proc. and Papers* **18:** 17–20.
22. Merrill, R. S., J. Savit and J. M. Tobias 1946, Certain biochemical changes in the DDT poisoned cockroach and their prevention by prolonged anesthesia, *J. Cell. Comp. Physiol.* **28:** 465–476.
23. Metcalf, R. L. and R. B. March 1951, (personal communication).
24. Monche, J. 1950, Acción del hexaclorociclohexano y del DDT sobre los procesos fosfatásicos, *Rev. esp. Fisiol.* **6:** 43–48.
25. Perry, A. S. 1950, (personal communication).
26. Perry, A. S. 1951, (personal communication).
27. Perry, A. S. and W. M. Hoskins 1950, The detoxification of DDT by resistant houseflies and inhibition of this process by piperonyl cyclonene, *Science* *111*: 600–601.
28. Pimentel, D., H. H. Schwardt, J. E. Dewey and L. B. Norton 1950, Studies on insecticide resistant house flies in New York, *Soap and Sanit. Chem.* **26:** 94–96.
29. Richards, A. G. and L. K. Cutkomp 1945, The cholinesterase of insect nerves, *J. Cell. Comp. Physiol.* **26:** 57–61.
30. Roeder, K. D. and E. A. Weiant 1946, The site of action of DDT in the cockroach, *Science* **103:** 304–306.

31. Sacktor, B. 1949, The effect of DDT and methoxychlor on cytochrome oxidase in two strains of house flies, *M. domestica* L., Rutgers Univ. Thesis.

32. Sacktor, B. 1950, A comparison of the cytochrome oxidase activity of two strains of houseflies, *J. Econ. Ent.* **43**: 832–838.

33. Sacktor, B. 1951, Some aspects of respiratory metabolism during metamorphosis of normal and DDT-resistant house flies, *Musca domestica* L., *Biol. Bull.* **100**: 229–243.

34. Sacktor, B. 1951 a, (personal communication).

35. Smith, J. B. 1897, The influence of environment on the life history of insects, *Gard. and Forest* **10**: 334.

36. Snyder, F. M. 1951, (personal communication).

37. Sternburg, J., and C. W. Kearns 1950, Degradation of DDT by resistant and susceptible strains of houseflies, *Ann. Ent. Soc. America* **43**: 444–458.

38. Sternburg, J.C., C. W. Kearns and W. N. Bruce 1950 a, Absorption and metabolism of DDT by resistant and susceptible houseflies, *J. Econ. Ent.* **43**: 214–219.

39. Sumerford, W. T., M. B. Goette, K. D. Quarterman and S. L. Schenck 1951, The potentiation of DDT against resistant houseflies by several structurally related compounds, *Science* **114**: 6–7.

40. Tobias, J. M. and J. J. Kollros 1946, Loci of action of DDT in the cockroach (*Periplaneta americana*), *Biol. Bull.* **91**: 247–255.

41. Tobias, J. M., J. J. Kollros and J. Savit 1946, Acetylcholine and related substances in the cockroach, fly, and crayfish and the effect of DDT, *J. Cell. Comp. Physiol.* **28**: 159–182.

42. Welsh, J. H. and H. T. Gordon 1947, The mode of action of certain insecticides on the arthropod nerve axon, *J. Cell. Comp. Physiol.* **30**: 147–172.

43. Wiesman, R. 1947, Untersuchungen über das physiologische Verhalten von *Musca domestica* L. verschiedener Provenienzen, *Schweiz. Ent. Gesell. Mitt.* **20**: 484–504.

44. Winteringham, F. P. W., P. M. Loveday and A. Harrison 1951, Resistance of houseflies to DDT, *Nature* **167**: 106–107.

45. Yeager, J. F. and S. C. Munson 1945, Physiological evidence of a site of action of DDT in an insect, *Science* **102**: 305–307.

FURTHER OBSERVATIONS ON THE USE OF STREPTOMYCIN AND PENICILLIN IN THE CULTIVATION OF *ENDAMOEBA HISTOLYTICA* FROM STOOLS

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This report deals with a comparative study of the effects of streptomycin and penicillin on the cultivation of *Endamoeba histolytica* from cysts present in stool specimens. In a previous communication, it was reported that the addition of streptomycin to culture media increased the number of positive ameba cultures. Additional data, to be presented in this paper, confirmed this finding and further demonstrated that the effect of the drug on amebic growth *in vitro* was not a specific one. A favorable influence on the cultivation of *E. histolytica* was also observed when media containing penicillin was used.

Methods: Fifty-four stools containing cysts of *E. histolytica* were obtained at The Mount Sinai Hospital, at the Tropical Disease Clinics of the Department of Health of the City of New York, and from patients of physicians in private practice. The cysts were found readily by microscopic examination of direct smears stained with dilute Lugol's solution. Specimens in which the nature of the cysts was doubtful or in which trophozoites of *E. histolytica* were found were not included.

The following technic of cultivation was used. Heavy fecal suspensions were prepared in 5 ml. of saline and were stored at 5°C. for 24 hours. The specimens were usually 30 to 72 hours old at the time of inoculation. Six tubes of media were set up for each specimen. Each contained a slant (7 ml. of Endamoeba medium (Difco) overlaid with 2.9 ml. of sterile horse serum-saline (1:6)) to which was added approximately 50 mgm. of sterile rice powder (Difco). Exactly 3 or 6 mgm. of streptomycin sulfate (Merck) were added to each of 2 tubes and 3,000 or 6,000 units of crystalline sodium penicillin G to 2 other tubes. The remaining tubes were kept as controls. The drugs were dissolved in small volumes of normal saline (0.1–0.3 ml.). The cultures were inoculated with 0.1 ml. of the fecal emulsion, were incubated at 37.5°C., and were examined daily for amebae, *Blastocystis hominis*, and rice grains for at least a week by removing small amounts of sediment with a capillary pipette.

The growth of the bacteria present in these cultures was evaluated by direct visual comparison of the turbidity of the serum-saline supernate, by noting the formation of gas, and the presence of colonies on the agar slants. In some experiments, the components of the bacterial flora were identified by subculturing on differential media. The sensitivity of the total bacterial population to each antibiotic was determined in a few cultures by preparing pour plates of nutrient agar with uniform inocula and various amounts of the drugs. The numbers of visible colonies were counted after incubation at 37.5°C. for 24 hours and were compared with those growing in the untreated plates.

Results: The effects of streptomycin and penicillin on the cultivation of *E. histolytica* are shown in Table I. Trophozoites appeared in 34 of the 54 cultures (63%) with added antibiotics, but in only 8 of the controls (14.8%). In 23 of the positive cultures, amebae were found in the tubes containing each of the drugs. However, in 9 (29%), trophozoites were seen only in the streptomycin media. On the other hand, amebae were seen exclusively in penicillin treated media twice. Only once was a control positive while the other tubes of the series were negative.

TABLE I

The Effect of Streptomycin and Penicillin on the Incidence of Positive Ameba Cultures

GROUP	TOTAL CULTURES	POSITIVE AMEBA CULTURES	
		Number	Per cent
Controls	54	8	14.8
Antibiotic treated (total)	54	34	63.0
1. Streptomycin	54	32	59.3
2. Penicillin	54	25	46.3

TABLE II

Distribution of Positive Culture Tubes for E. Histolytica According to Treatment

TREATMENT	TOTAL CULTURE TUBES	POSITIVE FOR TROPHOZOITES	
		Number	Per cent
Controls	103	9	8.7
Streptomycin (total)	108	45	41.6
1 mg/ml	54	20	37.0
2 mg/ml	54	25	46.3
Penicillin (total)	108	38	35.1
1000 u/ml	54	19	35.1
2000 u/ml	54	19	35.1

Trophozoites appeared in 45 of the 108 tubes (41%) containing streptomycin (Table II). In 14 of the 31 positive streptomycin cultures, they were seen simultaneously in each of the two concentrations of the drug. In the other 17, 6 positives occurred in the tubes containing 1 mg. per ml. while the remaining 11 occurred in the tubes containing 2 mg. per ml.

Of the 108 tubes containing penicillin, amebae were seen in 38 (35%). They appeared in both concentrations in 16 of the 25 positive cultures (64%). In 3 cultures, only the 1,000 units of penicillin per ml. tube provided a positive and in 3, only the 2,000 units of penicillin per ml. tube.

In the small number of control tubes in which trophozoites appeared, the latter grew poorly and usually disappeared within 72 hours. Heavy bacterial growth occurred in the liquid phase of the medium within 24 hours and the slants were often fragmented by large amounts of gas. The rice grains usually disappeared within 48 hours and *Blastocystis hominis* appeared in almost every tube (93%).

In the tubes containing antibiotics, amebic growth was often profuse and the trophozoites survived for 96 hours or longer. The agar slants and rice grains remained intact for 4 to 7 days. *Blastocystis hominis* appeared in 25% of the tubes containing streptomycin and in 40% of the tubes containing penicillin.

Both streptomycin and penicillin inhibited the bacterial flora associated with the ameba cultures. The degree of bacterial inhibition varied from culture to culture and was roughly proportional to the concentrations of the drugs used. In each instance, it was incomplete and temporary. As a rule, recovery of bacterial growth was noted within 96 hours after inoculation.

In 7 of the positive cultures no apparent difference was observed in the degree of bacteriostasis produced by each drug. In 17 cultures penicillin was more inhibitory while in 9, bacteriostasis was greatest in the tubes treated with streptomycin. These differences in bacteriostasis were also observed in pour plates containing various concentrations of penicillin and streptomycin and uniform inocula. A comparison of the intensity of bacterial inhibition and the time of appearance of trophozoites indicated that amebic growth occurred less frequently and usually after a longer latent period in the tubes showing the greatest bacteriostasis.

In some of the cultures treated with streptomycin, bacteria failed to grow on portions of the agar slants above the level of the supernate. A bacteriologic study of 4 such cultures yielded only anaerobic bacteria. In one culture, 2 anaerobes, *Clostridium perfringens* and *Bacteroides necrophorus*, were the only bacteria that could be recovered.

In the 20 cultures in which trophozoites were not found there was no consistent explanation for the failure of amebic growth. There was no evidence that these cultures were inoculated with fewer cysts than the positive cultures. In 6 instances, when a negative culture had been observed, subsequently passed stools from the same patient were cultured and again failed to yield trophozoites. In most of the negative cultures the degree of bacterial inhibition was similar to that noted in the positive ones. However, in 4 cultures bacterial growth was very sparse. In 5 of the negatives yeasts grew in abundance. However, similar structures were seen in 3 of the cultures in which good growth of trophozoites occurred. In 2 of the negatives, heavy growth of *P. aerugenosa* occurred in all the tubes. In one, the slants were heavily overgrown with a black mold.

Comment: Although the percentage of positive ameba cultures obtained with the aid of streptomycin in this series was lower than in our original series (62.9% as compared to 72%), the difference between the treated cultures and the controls was striking (Spingarn *et al.*, 1948). Of the combined total of 80 stools, streptomycin treated media yielded positive ameba cultures in 65% of the trials as compared to only 15% in the controls.

As shown above, the use of penicillin also favored the cultivation of *E. histolytica* and it was noted that excystation and amebic growth might occur only in penicillin-treated media. The somewhat better results obtained with streptomycin may have been due to the fact that penicillin produced a greater degree of bacteriostasis than streptomycin. That penicillin inhibits *Escherichia coli* and other coliform organisms under certain conditions of growth was demonstrated by Shwartzman (1944; 1946). Neither streptomycin nor penicillin has been found to possess a direct amebicidal action (Bradner *et al.*, 1951).

Our data seemed to indicate that the reduction in the total bacterial population produced by streptomycin and penicillin favored amebic growth *in vitro*. The use of these antibiotics retarded the rapid destruction of the nutrient properties of the medium by bacterial overgrowth and the disintegration of rice starch, an important stimulant of amebic growth. The presence of intact starch, furthermore, delayed the appearance of *Blastocystis hominis*, a fungus frequently appearing in ameba cultures (Dobell *et al.*, 1926).

Since only anaerobic bacteria were recovered from some of the cultures treated with streptomycin, a qualitative change in the flora as well as the apparent quantitative one may have aided in amebic cultivation. In this study, it was noted that Endamoeba medium overlaid with serum-saline supported the growth of *Clostridium perfringens* and *Bacteroides necrophorus* under aerobic conditions. This suggested that a similarity exists between the growth requirements of these bacteria and *E. histolytica*. In a comparison of the effect of bacterial strains on amebic excystation and growth, Chinn and his coworkers (1942) showed that only certain bacteria and fungi induced amebic growth from microisolated cysts. *Clostridium perfringens*, *Streptococcus fecalis*, and *Leptotrichia buccalis* were the most active in this respect among a group of 13 organisms. On the other hand, excystation was not obtained with 13 other strains including *E. coli*, *A. aerogenes*, and *P. aerugenosa*. These workers further showed that the addition of *Clostridium perfringens* to ameba cultures growing with a mixed bacterial flora enhanced amebic growth. Jacobs (1950) suggested that some common metabolite or constituent of bacteria such as *Clostridium perfringens* may be a growth factor for *E. histolytica*. Moreover, Shaffer and his coworkers (1948) were able to cultivate *E. histolytica* with an anaerobic streptobacillus and to maintain amebic growth in the absence of viable bacteria by using filtrates of this organism.

The cultivation of amebae from stools containing cysts was attended, in this series as in some others, by a high percentage of failures (38%). Dobell and Laidlaw (1926) stated that one cannot culture cysts from stools in 100% of experiments and that the result of an attempt could not be predicted with certainty due to the many unknown variables. According to Svenson and Linders (1934), cultures were often negative although examination of the sample from which they had been initiated revealed healthy looking cysts in large numbers. In some of our specimens, a negative culture may have been due to a high percentage of non-viable cysts, to extremely low cyst concentrations, to errors in cyst identification, or to antibiotic influences created by the rest of the microbiologic population. The latter may be a limiting factor in ameba cultivation in view of the demonstration that certain fungi and bacteria are capable of producing amebicidal as well as bactericidal substances (Bradner *et al.*, 1951; McCowen *et al.* 1951; Hewitt *et al.*, 1951; Spingarn *et al.* 1951).

SUMMARY

1. The use of media treated with streptomycin or penicillin increased the number of positive cultures of *E. histolytica* obtained from stool specimens containing cysts.

2. It seemed that the favorable influence on amebic cultivation was due to the inhibition of the mixed bacterial flora induced by these drugs.

3. Streptomycin suppressed the aerobic bacteria in some of the cultures and it was observed that slants of *Endamoeba* medium overlaid with serum-saline supported the growth of certain anaerobic bacteria under aerobic conditions.

4. In some instances, failure of amebic cultivation may have been related to antibiotic influences created by the microbiologic population.

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REFERENCES

Bradner, W. T. and Rawson, G. W.1951, *In vitro* method of screening amebicidal agents using the Phillips culture, *Science* **113**: 674.

Chinn, B. D., Jacobs, L., Reardon, L. V., and Rees, C. W. 1942, The influence of the bacterial flora on the cultivation of *Endamoeba histolytica*, *Am. J. Trop. Med.* **22**: 137.

Dobell, C. and Laidlaw, P. 1926, On the cultivation of *Endamoeba histolytica* and some other entozoic amebae, *Parasitol.* **18**: 267–283.

Hewitt, R., Wallace, W., and White, E. 1951, The effect of aureomycin on *Endamoeba Histolytica in vitro. Science* **112**: 144–146.

Jacobs, L. 1950, Oxidation-reduction potentials in the cultivation of *Endamoeba histolytica*, *Am. J. Trop. Med.* **50**: 803–815.

McCowen, M. C., Collender, M. E., and Lawlis, J. E. 1951, Fumagallen, (H-3), a new antibiotic with amebicidal properties, *Science* **113**: 202–203, Feb. 23.

Shaffer, J. G., Walton, J. G., and Frye, W. W. 1948, Studies on growth requirements of *Endamoeba histolytica*. II: Preliminary observation on cultivation of *E. histclytica* in a modified thioglycollate medium, *Am. J. Hygiene* **47**: 222.

Shwartzman, G. 1944, Inhibition of *E. coli* by penicillin, *Science* **100**: 477–478.

Shwartzman, G. 1946, Studies on nature of resistance of gram negative bacilli to penicillin; antagonistic and enhancing effects of amino acids, *J. Exper. Med.* **83**: 65–88.

Spingarn, C. and Edelman, M. 1948, The use of streptomycin in the cultivation of *Endamoeba histolytica* from stools, *Am. J. Trop. Med.* **28**: 825.

Spingarn, C., and Edelman, M. 1951, The effect of aureomycin hydrochloride on the growth of *Endamoeba histolytica in vitro*, *Am. J. Trop. Med.* **31**: 206–211.

Svenson, R. and Linders, L. 1934, Chances of detecting infections with intestinal protozoa, *Acta Med. Scand.* **81**: 267.

THE EXPERIMENTAL INFECTION OF *MACACA MULATTA* WITH HUMAN STRAINS OF *ENTAMOEBA HISTOLYTICA*[1]

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The studies of Dobell (1931), Kessel (1928), and others established the fact that the "histolytica-like" amebae found in monkeys are identical with *E. histolytica* in man. While it has been shown by many investigators that this parasite can be transmitted to a wide variety of hosts, in none of these can the infection be considered a normal one. Thus, dogs, kittens, rats, pigs, rabbits, guinea pigs and even chicks have been infected experimentally with *E. histolytica*. But in such cases infection is usually achieved only under special conditions. Also, the regular production of cysts is rarely seen, and it seems logical to assume that cyst-production here is good evidence of a normal host-parasite relationship For a proper understanding of the host-parasite relationships of *E. histolytica* the parasite must be studied in its normal host. Experimental infections in rodents and carnivores have provided information on the acute tissue-invasive stages of the disease, and have aided in the testing of amebicidal drugs, but apart from this they have attested only to the versatility of the parasite and have contributed no information towards an understanding of the host-parasite relationships in nondysenteric intestinal amebiasis in man.

The frequent occurrence of apparently asymptomatic *E. histolytica* infections in man is a well-established fact. However, it has been demonstrated clearly by Tobie (1940) and others, that strains of *E. histolytica* which produce asymptomatic human infections, can cause ulcerative colitis and dysentery when inoculated into experimental animals. Furthermore, Walker and Sellards (1913) have shown that a strain of *E. histolytica* which lives in one human host as an apparent commensal, when transferred to another human may cause amebic dysentery.

Because of the potential invasiveness of the so-called "carrier strains" of *E. histolytica* there has been much speculation about the host-parasite relationships of this organism in asymptomatic infections, and monkeys, being the only known normal hosts apart from man, should serve as ideal experimental animals for studies of such relationships. While amebiasis in monkeys has been studied, notably by Kessel (1928), Dobell (1931), Hegner *et al.* (1930), Hegner (1935), and Johnson (1941), the results have been somewhat conflicting. Accordingly, advantage was taken of a recent stay in India to infect rhesus monkeys (*Macaca mulatta*) with human strains of *E. histolytica* in an attempt to throw additional light on this problem.

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MATERIALS AND METHODS

Four young rhesus monkeys were used. They were purchased in the Calcutta market; their weights ranged from 1.75 to 2.75 pounds. Like all monkeys obtained from this source they harbored natural infections of *E. histolytica*, *Balantidium coli*, and several species of non-pathogenic intestinal amebae. The monkeys were kept in individual cages in quarters far removed from other monkeys kept on the premises. They were fed on gram (*Phaseolus aureus*), raw root vegetables and bananas. All food, with the exception of the bananas, was immersed in boiling water for at least five minutes before being fed, to destroy any *E. histolytica* which may have been present as a contamination.

Diiodohydroxyquinoline (Diodoquin) was used to eradicate the natural infections with *E. histolytica*. Six tablets (1.2 gm.) were administered daily to each monkey for eight consecutive days, making a total dosage of 9.6 gm. received by each animal. The drug was administered through a stomach tube as a suspension in water. This treatment was successful in eradicating the infections with *E. histolytica* and *Balantidium coli*. The non-pathogenic amebae, which included *E. coli*, *Iodamoeba bütschlii*, and *Endolimax nana* disappeared from the stool during, and for ten days following, the completion of treatment, but they then reappeared in the stool. At no time during the course of treatment were untoward effects due to the drug seen.

Three human strains of *E. histolytica* were used in the experimental infections. Strain C was obtained from a child seen as an outdoor patient. The child showed no signs and gave no symptoms attributable to the amebic infection, and was considered to be an asymptomatic carrier. The cysts ranged in size from approximately 10.0 to 13.5μ in diameter. This strain, in culture, showed sporadic cyst formation. Strain D was obtained from a patient seen in the outdoor clinic. This patient was suffering from acute amebic dysentery and the stool consisted of mucus and blood with relatively little fecal material. Numerous trophozoites of *E. histolytica*, many containing ingested red blood cells, were present. Strain E was obtained from a patient hospitalized for amebic dysentery. Sigmoidoscopy showed numerous typical amebic ulcerations of the sigmoid colon and rectum from which trophozoites of *E. histolytica* were recovered. This patient passed copious mushy stools with islets of blood and mucus, containing enormous numbers of both cysts and trophozoites of *E. histolytica*. The cysts ranged in size from approximately 11 to 16μ in diameter.

For post-mortem studies the animals were killed with ether and the large intestine, from above the ileo-cecal valve to the anus, removed. The bowel was laid open and the contents examined for the presence of mucus and blood. Saline-iodine wet-smear preparations were made of the lumen contents at two-inch intervals along the entire length of the large bowel, and examined for the presence of *E. histolytica* cysts and trophozoites. The fecal material was pushed aside and the wall examined for lesions; scrapings were then taken directly from the surface of the mucous membrane and examined as above for amebae. Finally, the bowel wall was washed in running water and examined carefully for evidence of amebic ulceration. When *E. histolytica* was found smears were fixed in Schaudinn's fluid and stained with iron hematoxylin. Tissue blocks taken for sectioning were fixed in Zenker's fluid and the sections stained with Mallory's ferric chloride hematoxylin. All tissues taken for sectioning were placed in fixative within one hour of the animal's death, and in most cases within 15 to 30 minutes of death.

EXPERIMENTAL PROTOCOLS

Monkey #16

This monkey weighed 2.25 lbs. at the start of the experiment.

Following completion of treatment a total of 21 stool examinations over a period of 25 days were all negative for *E. histolytica*. At this time the animal received 50 ml. stool suspension in water containing *E. histolytica* cysts of Strain C. The stool suspension contained approximately one cyst per low-power field in a wet-smear preparation, but only 25% of the cysts were 4-nucleated. A few cysts of *E. histolytica* appeared in the stools of the animal on the second day after inoculation. On the third day large numbers of cysts were passed. For the following 106 days of the experiment, stool examinations were made every week-day and *E. histolytica* was seen in 60% of the stools. The cysts were approximately the same size as those inoculated, ranging from about 10 to 13.5μ in diameter. At no time during the course of the infection did the animal show signs of disease: there was no diarrhea or dysentery, its appetite remained good and, at the close of the experiment, it weighed 3.5 lbs., showing a gain of 1.25 lbs. About 15 weeks after being infected with *E. histolytica*, the monkey was sacrificed and an examination made of the large bowel. The large bowel, from the proximal part of the ascending colon to the anus, contained both cysts and trophozoites of *E. histolytica*, but none was found in the cecum. The heaviest concentrations of the parasite were seen in the ascending colon. No blood or excess mucus was seen, and the mucous membrane looked normal and healthy throughout except for a small erosion of the mucous membrane of the ascending colon. The eroded area was removed for sectioning and study. Additional tissues for sectioning were also taken from the ascending colon, the descending colon, and the rectum.

A study of the tissue sections showed trophozoites and cysts of *E. histolytica* at all levels of the bowel sectioned. The trophozoites were small and delicate and in the fixed and stained state measured around 10μ. They were lying free in the lumen of the bowel usually surrounded by fecal material which separated them from the surface of the mucous membrane. Occasionally, a trophozoite, and sometimes a cyst, was seen lying in contact with the mucous membrane, but no trophozoite was ever seen penetrating the tissue or lying within the tissue, nor was there any evidence of tissue destruction by the amebae. Trophozoites of *E. coli* and *Iodamoeba bütschlii* also were seen on occasion lying in contact with the mucous membrane and rarely within the crypts. Occasionally bacteria were seen within the *E. histolytica* trophozoites but never were they as numerous or as consistently seen as in the trophozoites of *E. coli* and *I. bütschlii*.

The liver was negative for *E. histolytica*.

Monkey #17

This monkey weighed 1.75 lbs. at the start of the experiment.

Following the completion of treatment, 24 stool examinations over a period of 35 days were all negative for *E. histolytica*. The animal was considered free of infection and accordingly, was given, by stomach tube, 50 ml. aqueous stool suspension of monkey #16, which had previously been infected experimentally with *E. histolytica* strain C. The stool suspension showed an average of four cysts per low-power field in a wet-smear preparation and the majority were 4-nucleated. A stool examination 24 hours later, revealed the presence of *E. histolytica* cysts ranging in size from 10 to 13.5μ. These measurements agreed with the size range of strain C. For the following five days the cysts appeared in gradually increasing numbers; on the sixth day they were fewer in number, and on the seventh day, the stool was negative. For the succeeding 41 days the stools remained negative for *E. histolytica*. At that time 10 ml. of dysentery stool containing trophozoites of strain D were inoculated per rectum into this monkey. On the fourth day following inoculation, cysts of *E. histolytica* were passed in the stool. The cysts were somewhat larger than those of strain C, but not as big as those seen in strain E; they measured 11 to 14μ in diameter. Larger numbers of cysts continued to be passed for the first 15 days of infection. On the 16th day the stool was negative, and in the following month a total of 15 stool examinations showed only four positive for *E. histolytica*, and then only in very small numbers. At no time during the course of the first infection, or the reinfection, did the monkey show signs of disease; it never developed dysentery, maintained a good appetite and gained weight. When sacrificed, over seven weeks after the infection with strain D, it weighed 2.5 lbs., showing a gain of 0.75 lbs.

Immediately after it was killed the large bowel was removed and examined as for monkey #16. A careful search of the lumen contents and scrapings of the bowel wall did not reveal either cysts or trophozoites of *E. histolytica*. The proximal portion of the ascending colon showed several small erosions; these were removed and fixed for tissue sectioning. The distal portion of the ascending colon showed numerous diverticuli, some of which were partially occluded and forming cysts. The cyst contents were examined but found negative for amebae. One cyst was taken for tissue sectioning. In the descending colon a small superficial ulcer was seen; this was removed for sectioning. A study of the tissue sections showed neither cysts nor trophozoites of *E. histolytica* in the lumen contents or the tissues of the bowel wall.

The liver was negative for *E. histolytica*.

Monkey #18

This monkey weighed 2.75 lbs. at the start of the experiment.

Following the completion of treatment, a total of 59 stool examinations over a period of 78 days were all negative for *E. histolytica*. At that time this animal received by stomach tube 50 ml. of aqueous stool suspension containing *E. histolytica* cysts of strain E. The fecal suspension showed one to two cysts per low-power field in a wet-smear preparation, and the majority were 4-nucleated.

The next day about the same number of cysts of strain E were administered in a similar manner. A stool examination the following day revealed cysts of *E. histolytica* measuring 11–16μ in diameter, which was the size range for cysts of strain E. During the six weeks after infection that this animal was followed, it consistently passed cysts and trophozoites of *E. histolytica*, usually in large numbers. At no time during the course of the infection was there any evidence of disease. There were no signs of diarrhea or dysentery and the animal retained a good appetite. Although it gained weight, this was comparatively small as compared with the other monkeys: when sacrificed it showed a weight of 3 lbs. or only 0.25 lbs. gain over its original weight.

This animal was sacrificed about six weeks after infection and the large bowel removed and examined as described for monkey #16. No blood or excess mucus was seen anywhere in the bowel. Cysts and trophozoites of *E. histolytica* were found in the lumen contents and scrapings of the bowel wall in all levels from the distal two-thirds of the ascending colon to the anus. The amebae were present in large numbers and were particularly numerous in the descending colon. The cecum and proximal third of the ascending colon were free of amebae. The mucous membrane was microscopically normal throughout. Two pieces of tissue were taken from the descending colon, and one each from the cecum and rectum. A study of the tissue sections showed large numbers of *E. histolytica* cysts and trophozoites lying free in the lumen contents of the bowel and some adjacent to the mucous membrane. There was no evidence of tissue invasion by the trophozoites and in no instance was an ameba found lying within the tissue. Some of the trophozoites showed a few ingested bacteria but, as noted above, they were never as numerous or as consistently present as in the trophozoites of *E. coli* and *Iodamoeba bütschlii*.

The liver was negative for amebae.

Monkey #19

This monkey weighed 1.75 lbs. at the start of the experiment.

Following the completion of treatment a total of 71 stool examinations over a period of 93 days were negative for *E. histolytica*. On the 93rd day after completion of treatment the animal was given per rectum 20 ml. of dysenteric stool containing numerous active trophozoites of *E. histolytica* strain E; about one-half of this material was retained. Four days later, 10 ml. of the same material were given in a similar manner and all was retained by the monkey. On the fifth day after the second inoculation, cysts of *E. histolytica* measuring 11 to 16μ appeared in the stool and were passed more or less regularly during the following six weeks that the animal lived. During this period the monkey retained a good appetite and showed no signs of being affected in any way by the infection with *E. histolytica*; when sacrificed, it weighed 3.25 lbs., showing a gain of 0.75 lbs.

Approximately six weeks after the infection was induced the monkey was sacrificed and the large bowel removed and examined as described for monkey #16. Trophozoites and cysts of *E. histolytica* were found in all areas from the distal half of the transverse colon to the anus. No amebae were found in the ascending colon or cecum. No blood or excess mucus could be seen anywhere in the bowel and no lesions of the mucous membrane were seen. Pieces of tissue were removed from the sigmoid colon and rectum as these were areas where the greatest concentration of amebae were found. A study of the tissue sections showed that the mucous membrane was intact throughout with no evidence of tissue invasion by the amebae. Very few amebae were found in the sectioned material because the lumen contents, in which the amebae are found, had to a large extent broken away from the bowel wall during preparation of the slides.

DISCUSSION

Four young *Macaca mulatta* monkeys were inoculated with three human strains of *E. histolytica* and all became infected. However, in no case was there evidence of host-tissue invasion by the parasite. It is possible that because of the relatively small amount of bowel that was sectioned and examined, amebae invading the tissue may have been missed. However, a study of sections of the bowel in all the infected monkeys revealed amebae in the lumen but never within the tissues. Furthermore, in some sections the amebae were very numerous indeed[3] indicating that they were growing well and multiplying rapidly. It seems most probable, therefore, that the amebae were living in the monkey hosts as commensals. It is of interest to note that cysts of strain C which were from an asymptomatic case, were notably smaller than the cysts of strain E which came from a case of acute amebic dysentery. However, when established in monkey hosts there was no alteration in the size of the cysts produced by each strain, even though strain E was now living as a commensal.

The results of other studies on amebiasis in monkeys must now be reviewed.

Kessel (1928) reported the experimental infection of two rhesus monkeys with *E. histolytica* cysts from a human stool. He sacrificed them 12 and 21 days, respectively, after inoculation. An examination (which included tissue sectioning) of the large bowel showed no evidence of tissue invasion by the amebae. Dobell (1931) reported an experimental infection of *Macaca sinica* with cysts of a human strain of *E. histolytica*. The infection persisted for 16 months during which time the monkey remained healthy and grew rapidly. At no time did it show dysentery and when examined post-mortem showed no trace of amebic ulceration in the large bowel. The same author also reported an experimental infection of a rhesus monkey with a human strain of *E. histolytica* growing in culture. During the five weeks that this infection persisted it produced no signs of disease in the monkey host. Hegner *et al.* (1932) reported on the most extensive study yet made on the experimental infection of monkeys with human strains of *E. histolytica*. They inoculated one red spider monkey (*Ateles geoffroyi*), three white-faced monkeys (*Cebus capucinus capucinus*), 15 brown howler monkeys (*Alouatta palliata palliata*), one night monkey (*Aotus zonalis*), two marmosets (*Leontocebus geoffroyi*), and two black spider monkeys (*Ateles dariensis*). Of these, the red spider monkey, 14 of the 15 brown howler monkeys, and one of the two marmosets became infected with *E. histolytica*. Post-mortem studies of the infected monkeys showed tissue invasion by the amebae in all but four of the brown howlers. In some cases microscopic lesions were not apparent but on sectioning, amebae could be seen invading the tissue. Hegner (1935) later reported the experimental infection of four rhesus monkeys with human strains of *E. histolytica*. From the context of the report it is not possible to interpret the experiments clearly. However, the results indicate that the four monkeys were infected either with cultures or with cysts of a human strain of *E. histolytica*. They were sacrificed 12, 18, 19 and 36 days respectively after inoculation was induced. Post-mortem examinations failed to reveal evidence of tissue invasion.

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[3] In monkey #18 as many as seven trophozoites per oil-immersion field were seen.

With regard to natural infections with *E. histolytica* in monkeys, Eichorn and Gallagher (1916) reported a spontaneous outbreak of amebic dysentery in which nine spider monkeys died of the disease. McCarrison (1919) reported four cases of spontaneous amebic dysentery in *Macaca mulatta* which had been kept on a polished rice diet. Kessel (1928) found that in six rhesus monkeys naturally infected with *E. histolytica* and autopsied for study, two showed patches of excess mucus overlying the mucous membrane of the large bowel; tissue sections from these areas showed no evidence of ulceration but revealed isolated amebae wandering through the tissue. The remaining four monkeys gave no evidence of tissue invasion by the amebae. Dobell (1931) described a natural *E. histolytica* infection in a rhesus monkey which he followed for four years, during which time the animal never showed signs of dysentery and at post-mortem revealed no lesions of the bowel wall. Ratcliffe (1931) described an epidemic of amebic dysentery in *Ateles* and *Lagothrix* monkeys and stated that these monkeys developed the infection after exposure to old world monkeys who were carriers of *E. histolytica*. Johnson (1941) reported on the post-mortem findings in four red spider monkeys, three black spider monkeys and four rhesus monkeys, all naturally infected with *E. histolytica*. He found that in six of the seven spider monkeys and two of the four rhesus monkeys there was evidence of tissue invasion. Johnson (loc. cit.) indicates that rhesus monkeys are not as susceptible to tissue invasion as spider monkeys, as evidenced by the lower percentage showing tissue invasion and the lesser degree of tissue damage in those showing such invasion. Bond *et al.* (1946) reported autopsy findings on five rhesus monkeys harboring natural infections with *E. histolytica*. They describe chronic inflammatory changes in the bowel, with foci of interstitial hemorrhage and shallow ulcerations. The character of the described lesions, the fact that these monkeys had previously been treated with drugs which were being tested for their amebicidal properties, and the absence of amebae in the vicinity of the lesions leaves little doubt that the lesions had nothing to do with the amebic infections. We have seen similar lesions in monkeys dying of an overdose of emetine-bismuth-iodide tablets. These authors state that some amebae were found lying in and invading the tissue. However, in view of the previous insult to the mucous membrane by drugs, this observation cannot be considered to have much significance.

To summarize, the results of Kessel, Dobell, Hegner, and of the present investigation show that of 12 monkeys experimentally infected with human strains of

PLATE 1

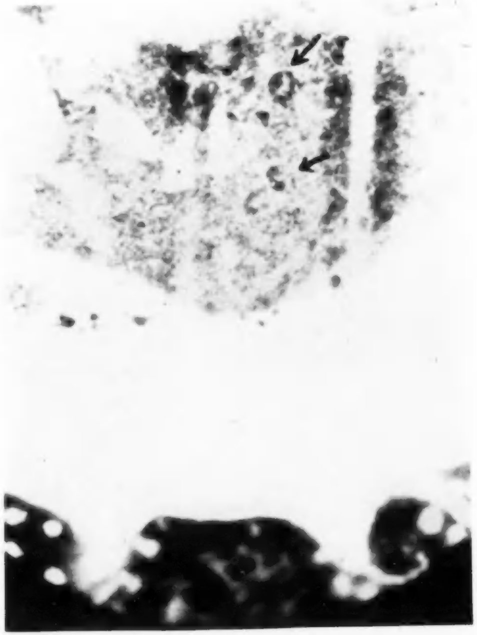

FIG. 1

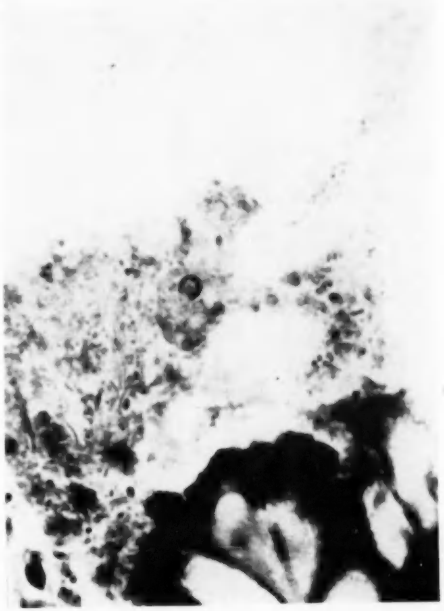

FIG. 2

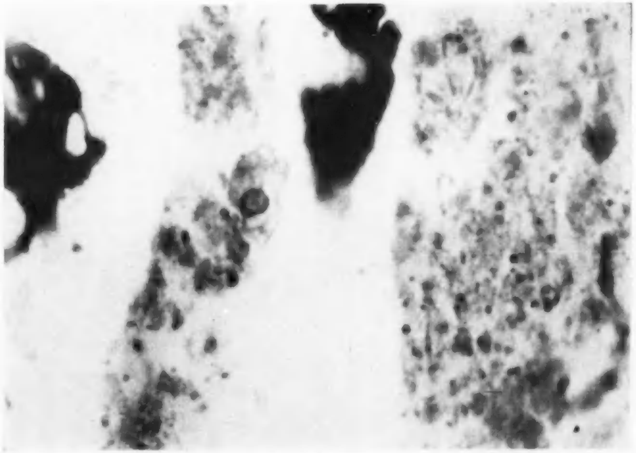

Fig. 3

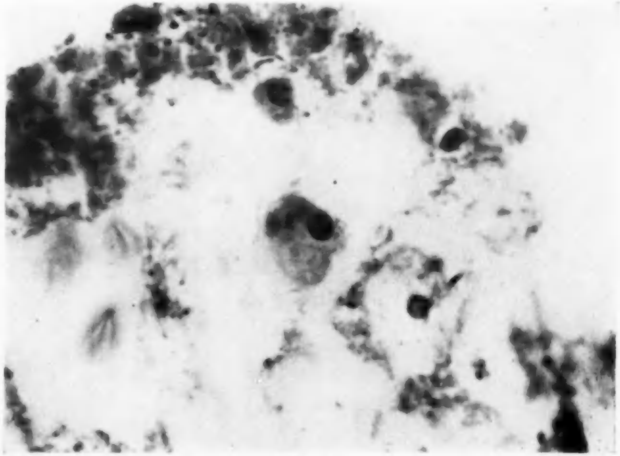

Fig. 4

Photomicrographs of tissue sections showing trophozoites in the lumen of the colon of *Macaca mulatta* monkey #18 experimentally infected with a human strain of *E. histolytica*.

Fig. 1. Section showing two trophozoites of *E. histolytica* and their position relative to the mucus membrane. The lumen contents have become somewhat separated from the bowel wall.

Fig. 2. Section showing a trophozoite of *E. histolytica* lying in the mouth of a crypt of Lieberkühn.

Fig. 3. Section showing a trophozoite of *E. histolytica* lying in the bowel lumen surrounded by fecal debris.

Fig. 4. Section showing three trophozoites lying among fecal debris in the lumen of the bowel. One of the amebae shows ingested bacteria.

E. histolytica, not one showed evidence of tissue invasion by the parasite. On the other hand, the work of Hegner *et al.* (1932), demonstrated that 12 of 16 monkeys experimentally infected with human strains of *E. histolytica* gave evidence of tissue invasion, and in some cases frank dysentery. The marked invasiveness of the *E. histolytica* infections in monkeys found by Hegner *et al.* is in sharp contrast to the lack of invasion seen by Dobell (1931), Kessel (1928), Hegner (1935), and the present study. The explanation of the conflicting results may be found in the species of monkeys used. Thus, all 12 of the monkeys used by the workers who found no evidence of tissue invasion, were old world monkeys of the genus *Macaca*, whereas Hegner *et al.* (1932) used new world monkeys. There is other evidence to suggest that amebae are more invasive in new world monkeys than in old world species of *Macaca*: Johnson (1941) in his study of natural infections of *E. histolytica* in monkeys, observed that rhesus monkeys appeared to be less susceptible to tissue invasion by amebae than the new world species he studied. And we have the significant statement of Ratcliffe (1931) that an epidemic of amebic dysentery among *Ateles* and *Lagothrix* monkeys was apparently initiated by contact with old world monkeys who were carriers of *E. histolytica*.

It would appear that old world monkeys of the genus *Macaca* are older and better adapted hosts for the parasite *E. histolytica*. And, while this parasite can and does invade the tissues of these monkeys, this seems to happen only infrequently, and it is difficult to avoid the conclusion that in the majority of infections, *E. histolytica* lives in the lumen of the large bowel of the species of *Macaca* as a facultative commensal.

In man, it is well known that in the majority of infections dysentery does not occur. Whether this is due to minimal tissue damage or to the organism living as a commensal cannot at present be definitely stated. The only investigation bearing directly on the problem is that of Faust (1941). He reported on a study of 202 autopsy cases in which the large bowel was examined within four hours after death. Thirteen, or 6.4%, showed the presence of *E. histolytica*. Of the 13, he states: "amebic lesions were seen in seven." In five, trophozoites were removed from the centre of the lesions, and in two, they were found near the lesions. Because it is not mentioned, one assumes that amebae were never seen within the lesions in the tissue sections, and although Faust states that "amebae were demonstrated in stained sections from only two cases," there is no reference to the relative positions of the lesions and the parasites. Amebae living in the bowel as commensals can be seen in stained sections, and in the present study, trophozoites of *E. coli* have been seen lying against the healthy cells of the mucous membrane and, in some cases, buried in the crypts of Lieberkühn.

Faust (loc. cit.) suggests that the examination by various technics of material taken from or near lesions is a more efficient method of demonstrating the causative organism in amebic infections at autopsy than is the study of tissue sections. While this is true, it does not prove that any amebae so found are necessarily associated with lesions present in the bowel. There is no reason why amebae present in the lumen of the bowel and living as commensals should not be found in material overlying small lesions regardless of the etiology of these lesions. And the mere fact of their presence in a superficial smear does not establish their role as the causative agents. Furthermore, from information available on monkey infections, the early stages of tissue invasion in amebiasis are characterized by amebae wandering through the mucous membrane without causing noteworthy tissue disarrangement. And only when the amebae become numerous enough to set up a necrosis of tissue are lesions formed. There is, therefore, at least some doubt that the "amebic lesions" described by Faust were in reality caused by the amebae.

For the purpose of this discussion, the better known natural hosts of *E. histolytica* may be divided into three groups. First, there is the group including the new world monkeys which, judging from the findings of Hegner *et al.* (1932), apparently in nature rarely harbor *E. histolytica*, but which take the infection readily and in the majority of infections, show evidence of tissue invasion and not infrequently the development of dysentery. Secondly, there is a group represented by the species of *Macaca* which in nature frequently harbors *E. histolytica* (Hegner and Chu, (1930)), is readily infected with the parasite but shows evidence of tissue invasion infrequently, and develops dysentery only rarely. And, finally, we have the group of human infections, which shows a relatively high infection rate, but a low dysentery rate in most parts of the world.[4] The degree of tissue invasion in the human nondysenteric intestinal amebiasis cases is, of course, not known, but a comparison of the clinical manifestations of amebiasis in these three groups would indicate that the course of infection in the old world monkeys more nearly resembles that seen in humans than does the course of infection in new world monkeys. However, the question of whether *E. histolytica* can live in the human bowel as a commensal, as it appears to do in the *Macaca* ispp., must at present remain unanswered. Further studies along the lines carried out by Faust (1941), with emphasis on study of tissue sections, would be most useful in helping to solve this problem.

ACKNOWLEDGEMENTS

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REFERENCES

BOND, V. P., BOSTICK, W., HANSEN, E. L., AND ANDERSON, H. H. 1946, Pathologic study of natural amebic infection in macaques, *Am. J. Trop. Med.* **26**: 625–629.

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[4] Even in such a highly endemic area as Calcutta, recent personal observations have revealed that, while amebic dysentery is not rare, the number of cases forms but a small percentage of the total number of cases of amebiasis present in that area.

Dobell, C. 1931, Researches on the intestinal protozoa of monkeys and man. IV. An experimental study of the *histolytica*-like species of *Entamoeba* living naturally in macaques, *Paras.* **23:** 1–71.

Eichhorn, A., and Gallagher, B. 1916, Spontaneous amebic dysentery in monkeys, *J. Inf. Dis.* **19:** 395–407.

Faust, E. C. 1941, Amebiasis in the New Orleans population as revealed by autopsy examination of accident cases, *Am. J. Trop. Med.* **21:** 35–48.

Hegner, R. 1935, Absence of tissue invasion in monkey carriers of *Endamoeba histolytica*, *Am. J. Trop. Med.* **15:** 41–43.

Hegner, R., and Chu, H. J. 1930, A comparative study of the intestinal protozoa of wild monkeys and man, *Am. J. Hyg.* **12:** 62–108.

Hegner, R., Johnson, C. M., and Stabler, R. 1932, Host parasite relations in experimental amoebiasis in monkeys in Panama, *Am. J. Hyg.* **15:** 394–443.

Johnson, C. M. 1941, Observations on natural infections of *Endamoeba histolytica* in *Ateles* and *Rhesus* monkeys, *Am. J. Trop. Med.* **21:** 49–58.

Kessel, J. 1928, Intestinal protozoa of monkeys, *Univ. Calif. Publ., Zool.* **31:** 275–306.

McCarrison, R. 1919, Pathogenesis of deficiency disease. VII. The effects of autoclaved rice dietaries on the gastro-intestinal tract of monkeys, *Indian J. Med. Res.* **7:** 283

Ratcliffe, H. L. 1931, A comparative study of amoebiasis in man, monkeys and cats, with special reference to the formation of the early lesions, *Am. J. Hyg.* **14:** 337–352.

Tobie, J. E. 1940, Pathogenicity of "carrier" strains of *Endamoeba histolytica* in the experimental dog, *Proc. Soc. Exp. Biol. Med.* **45:** 691–693.

Walker, E. L., and Sellards, A. W. 1913, Experimental endamoebic dysentery, *Philipp. J. Sci.*, (*B*) **8:** 255–331.

AUREOMYCIN IN AMEBIC LIVER ABSCESS

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The value of the antibacterial approach to the treatment of acute amebic dysentery has been well established (Armstrong *et al.*, 1950; Hargreaves, 1945; Most *et al.*, 1950). We (Armstrong *et al.*, 1950a) and others (Gutch, 1950; Halowani *et al.*, 1950; Hughes, 1950; McVay *et al.*, 1950), have shown that Aureomycin is among the most effective of this type of therapy. The action of such drugs is almost certainly to a large extent on the bacterial flora of the bowel, but there is a suggestion that there may be some direct action by the antibiotic on the protozoon. The assessment of this by *in vitro* experiment is difficult (Jones, 1950), for *Endamoeba histolytica* cannot be cultivated without the presence of living organisms either the fecal bacteria inoculated from the stool, or exceptionally other organisms such as *Trypanosoma cruzi* (Phillips *et al.*, 1950; Phillips, 1950). These associated organisms may be affected by the antibiotic and the mode of action of the drug becomes obscure. However, Balamuth and Brent (1949) showed that prodigiosin, an antibiotic prepared from *Serratia marcescens* had a direct amebicidal action. Fuller and Faust (1949) found that there is a concentration of Aureomycin which is amebicidal without being lethal to the bacteria in their cultures. By a process of seeding treated amebae in tubes with untreated bacteria, Hewitt, Wallace and White (1950) demonstrated that Aureomycin was amebicidal in a dilution of 1:1000 whereas at this dilution emetine was not. Watt and Van der Griff (1950) showed that Aureomycin had a greater amebicidal effect *in vitro* than had various polymyxins and Circulin. We had noted that there is, *in vivo*, an apparent interference with division, for under treatment with Aureomycin giant amebae appeared in the feces just prior to their disappearance. We can also confirm the findings of Hewitt *et al.* (1950).

The only place where amebae grow without the presence of microorganisms is in metastatic abscesses. Here they are apparently dependent on the human tissues to supply the factors necessary for growth usually supplied by bacteria. As Aureomycin is absorbed systemically (Harrell *et al.*, 1949), we felt that it would be of vzlue to try Aureomycin in liver abscess. Bordes and his colleagues (1950) reported the trial of this drug in two cases of amebic hepatitis, with one success and one failure. Hall (1951) reported four cases of hepatitis given this therapy with somewhat inconclusive results.

MATERIAL AND METHOD

Five cases were selected, all African males presenting clinically as amebic liver abscess. In four of these characteristic pus was aspirated from the liver during the course of treatment and in the fifth case the abscess ruptured into the lung and the patient coughed up "anchovy sauce" sputum.

After initial investigation of each patient, Aureomycin was given in a dose of 500 mgm. 6 hourly for 10 days, a total of 20 gm. Except for analgesics, no other drugs were given. Aspiration of the liver was done 2–5 days after the start of treatment and repeated when clinically indicated.

CASE HISTORIES

Case 1: A native male aged 31 years was admitted on 12/4/50 complaining of constant pain in his right lower chest and a cough with thin blood stained sputum. There was no history of any previous illness.

On examination he appeared wasted, his mucosae were pale, his temperature was 101°F and his pulse 112 per minute. The right lower chest was "bulging"; movement was diminished, the percussion note much impaired and air-entry absent at the right base. The liver was one-finger enlarged and tender. Intercostal tenderness was present.

Investigations:

Blood Count: Hb—8.55 gm. per cent; R.B.C.—3.1 million per c.mm.; W.B.C. —13.4 thousand per c.mm.

Radiograph of Chest: "The position of the right diaphragm is not distinctly seen, but is probably raised. There also appears to be either consolidation with atelectasis of the right middle lobe, or possibly, an interlobar effusion."

Sputum Examination: did not show amebae or tubercle bacilli.

On 17/4/50 the patient coughed up "anchovy sauce" sputum. Treatment with Aureomycin was begun on 18/4/50.

Progress: The patient continued to cough up purulent sputum which gradually decreased in amount and had ceased by 11/5/50. Needling through the eighth intercostal space in the anterior axillary line on 21/4/50 produced only a little blood. A further radiograph showed slight clearing of the right base, but on 22/5/50 the right diaphragm was still raised and irregular. Mottling of the right lower lobe was present. Symptomatically, however, the patient was much improved.

At his request, he was discharged on 24/5/50, for domestic reasons, with instructions to return in one week. He has failed to report.

Case 2: An African male aged 28 years was admitted on 10/7/50 complaining of pain in the right lower chest and right hypochondrium for two months. He had an unproductive cough which caused pain in the right chest. There was no previous history of dysentery.

On examination the temperature was 100°F and the pulse rate 102. There was diminished movement of the chest on the right, the percussion note was impaired and the air entry weak at the right base. He was exquisitely tender in the right lower intercostal spaces. The liver was two-fingers enlarged and very tender.

Investigations:

Blood Count: Hb.—13.3 gm per cent; R.B.C.—4.1 million per c.mm.; W.B.C. —6.6 thousand per c.mm.

Radiograph of Chest: "Well marked elevation of the right diaphragm, with linear atelectasis in the post-basic segment of the right lower lobe."

Aureomycin 500 mgm. 6 hourly was given for 10 days starting on the 13/7/50.

The liver was aspirated through the lower right intercostal spaces on 17/7/50, 19/7/50 and 22/7/50 during the course of the Aureomycin therapy with the removal of a total of 500 cc. of typical "anchovy sauce" pus which contained occasional pus cells and much cell debris. No amebae were seen in the pus and culture was sterile.

Progress: A slight initial improvement occurred, but towards the end of the treatment the patient's symptoms were becoming more severe and the physical signs were the same as on admission. On 22/7/50 the patient's temperature was 102°F and he complained that he had been unable to sleep the previous night because of severe pain. At this stage a course of emetine was begun with a rapid amelioration of symptoms. In all, two courses of emetine were given, the first 10 grains in 10 days and the second 6 grains in 6 days, separated by 10 days during which time chloroquin diphosphate was given. Further liver aspirations were carried out.

The patient was discharged after his second course of emetine, on 16/8/50, symptom and sign free.

Case 3: A male African aged 36 years was admitted on 14/8/50 complaining of pain in the right hypochondrium for two months. Frequent watery stools had been passed for the last seven days. There was no previous history of dysentery.

On examination his temperature was 100°F and his pulse 100 per minute. The liver was found to be four-fingers enlarged, smooth and very tender. Marked tenderness was present in the lower right intercostal spaces. No other abnormal physical signs were found.

Investigations:

Blood Count: W.B.C.—18 thousand per c.mm.; Polynuclears—60 per cent; Lymphocytes—35 per cent; Monocytes—1 per cent; Eosinophils—4 per cent.

Radiograph of Chest: "The right diaphragm does not appear to be raised, but there is a small hump anteriorly which may represent sub-diaphragmatic pathology. Lung fields clear."

Aureomycin was again given for 10 days (500 mgm. 6 hourly). On 19/8/50 180 cc. of "anchovy" pus were aspirated from the liver in the right hypochondrium. Microscopically the fluid contained a few pus and red blood cells and much cell debris. No amebae were seen and culture was sterile.

Progress: The patient's clinical condition changed little except for some decrease in his liver tenderness. He continued to run a remittent fever up to 100–101°F and his liver did not decrease in size. On the 29/8/50 (5 days after his course of Aureomycin) he complained of increasing pain in his right lower chest. On 30/8/50 a course of emetine was started. He received 1 grain of emetine on 10 successive days, a 14 day course of chloroquin diphosphate and a further 6 grains of emetine. His response was rapid and complete. A further 300 cc. of pus were aspirated from his liver on 31/8/50.

He was discharged from hospital symptom and sign free on 25/9/50.

Case 4: A male African aged 18 years was admitted to hospital on 25/9/50 complaining of pain in the right hypochondrium for 14 days. He stated that three months previously he had had an attack of diarrhea with blood and mucus which cleared up spontaneously.

On examination the temperature was 100°F and the pulse 90 per minute. His liver was three-fingers enlarged, smooth and extremely tender. There was exquisite tenderness in the right lower intercostal spaces. The percussion note was impaired and air entry diminished at the right base.

Investigations:

Blood Count: W.B.C.—10 thousand per c.mm.; Polynuclears—72 per cent; Lymphocytes—22 per cent; Monocytes—1 per cent; Eosinophils—5 per cent.

Radiograph of Chest: "The right diaphragm is raised and the appearance compatible with subdiaphragmatic pathology."

Starting on 26/9/50 500 mgm. of Aureomycin 6 hourly were given for 10 days.

Progress: No improvement occurred until 30/9/50 when 1200 cc. of "anchovy" pus were aspirated from the liver through the eighth intercostal space in the mid-axillary line. Microscopically the pus contained scanty pus and red blood cells with much cell debris, but no amebae were seen. Culture was sterile. After this, the liver decreased somewhat in size and gradually became non-tender. The intercostal tenderness also slowly disappeared. A further aspiration was attempted on 5/10/50, but no pus was obtained. The temperature had become normal 4 days after admission. The patient was observed for a further 16 days in hospital, at the end of which period the liver was two-fingers enlarged, non-tender and the intercostal tenderness had completely disappeared. A radiograph of the chest showed decreased height of the right diaphragm.

The patient felt very well and he was discharged on 21/10/50 with instructions to report again in 2 weeks. He has not returned to the hospital.

Case 5: A male African aged 49 years was admitted on 11/9/50 complaining of a painful swelling in the left hypochondrium and epigastrium for 7 days. He had had diarrhea with blood and mucus for the past 14 days.

On examination, the temperature was 98°F and the pulse 92 per minute. There was a visible mass in his left hypochondrium about the size of an orange. On palpation, the mass appeared to be continuous with the liver which was palpable in the epigastrium and left hypochondrium. The mass was extremely tender.

Investigations:

Blood Count: W.B.C.—18.8 thousand per c.mm.; Polynuclears—70 per cent; Lymphocytes—26 per cent; Monocytes—4 per cent.

Radiograph of Chest: Normal.

Stool Examination: Showed pus, blood and mucus and vegetative forms of *E. histolytica.*

Aureomycin was given for 10 days in a dosage of 500 mgm. 6 hourly.

Progress: Aspiration of the mass on 23/9/50 produced 6 ccs. of "anchovy" pus. 16 days after admission to hospital the patient was symptom free, the liver was no longer enlarged, but there was some induration in the left hypochondrium. The patient was discharged on 27/9/50 and instructed to return in one month's time. He returned 6 weeks later and was re-admitted. The swelling in his left hypochrondrium had recurred and was larger than when originally seen. He was treated with two courses of emetine separated by a course of chloroquin diphosphate. 150 ccs. of "anchovy" pus were aspirated from the mass. Response was complete and the patient was discharged on 6/11/50. 5 months later he had not returned to hospital.

RESULTS

The results obtained are summarised in Table 1. In two of the five cases treated (Cases 2 and 3), Aureomycin had no demonstrable effect on the course of the disease, while emetine and chloroquin diphosphate produced a rapid cure.

In Case 1, in which the liver abscess had ruptured into the lung, a good response, symptomatically, was obtained, but a radiograph showed residual changes both in the right lung and the right cupola of the diaphragm. The residual abnormality of the diaphragm is probably not significant (Todd, 1950) and without follow-up it is impossible to assess the importance of the radiographic signs in the right lung field. Unfortunately, the further progress of this patient is not known.

TABLE 1

Results of treatment of five cases of amoebic liver abscess with aureomycin

CASE NO.	SEX	AGE YEARS	CLINICAL DESCRIPTION	RESPONSE TO AUREOMYCIN	RESPONSE TO EMETINE
1.	Male	31	Amoebic liver abscess. Ruptured into right lung	Incomplete	Not given
2.	Male	28	Right lobe amebic liver abscess	None	Good
3.	Male	36	Right lobe amebic liver abscess	None	Good
4.	Male	18	Right lobe amebic liver abscess	Partial	Not given
5.	Male	49	Left lobe amebic liver abscess pointing in left hypochondrium	Incomplete. Relapse in 6 weeks.	Good

In Case 4, the response to Aureomycin was fair, but the liver was still clinically enlarged on discharge from hospital and as the patient failed to return for follow-up, no definite conclusion can be drawn.

A successful result with Aureomycin seemed to have been obtained in Case 5, but the liver abscess had recurred within 6 weeks of discharge, although the associated amebic dysentery was apparently cured.

DISCUSSION

Although only a small series of cases have been treated with Aureomycin, the results appear to be definitely inferior to those obtained with emetine alone or a combination of emetine and chloroquin diphosphate. In two of the series (Case 1 and 4) a cure is possible; in two (Cases 2 and 3) there was no response to Aureomycin while orthodox treatment produced a rapid response; in one case (Case 5) relapse followed within six weeks of apparent cure.

As a basis of comparison the results obtained in 77 consecutive cases of amebic liver abscess in African males admitted during 1950 may be quoted. Cases complicated by secondary infection, severe anemia, severe amebic dysentery or rupture into serous cavities are not included. They were treated with two courses of emetine, one of ten grains and one of six grains, separated by a 10–14 day course of chloroquin diphosphate and aspiration of amebic pus was carried out in all cases except some of those in which "anchovy" sputum was coughed up. All cases recovered, response to treatment was prompt and there have been no known relapses five months after discharge of the last case. During the same period seven cases were treated with emetine only in two courses, one of ten grains and one of six grains separated by 10–14 days without specific treatment. The results in these cases were similar to those with emetine and chloroquin diphosphate.

One is tempted to wonder if the improvement obtained in some of the cases treated with Aureomycin was entirely due to drainage of the abscess and the anti-bacterial properties of the drug and not to any amebicidal action of Aureomycin in the liver. Before the use of ipecacuanha and emetine some cases of "tropical abscess" recovered with surgical drainage and in many of those who died secondary infection of the abscess cavity must have been a potent cause of death. Clinical trial of this hypothesis is not possible for humanitarian reasons. It may be argued that the lack of response of amebic liver abscess to Aureomycin is to some extent due to its action on the bacterial flora of the bowel.

It would seem that Aureomycin is definitely inferior to accepted drugs in the treatment of amebic liver abscess, and that there is no good resaon for its use in this condition except, possibly, for the treatment of associated bowel lesions due to *E. histolytica*.

SUMMARY

Five cases of amebic liver abscess in male Africans in which characteristic pus was obtained were treated with Aureomycin in a dose of 500 mgm. 6 hourly for 10 days.

In two cases there was no response, in two cases possibly some response, and in one case an apparently good result was followed by recurrence in six weeks.

The results have been compared with those obtained in similar cases with emetine and chloroquin diphosphate and it is concluded that Aureomycin is a much less effective drug.

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REFERENCES

ARMSTRONG, T. G., MAROT, J. T., AND ELSDON-DEW, R.: Studies in amoebiasis: The effect of anti-bacterial drugs. *S. A. M. J.*, **24:** 121, 1950.

ARMSTRONG, T. G., WILMOT, A. J., AND ELSDON-DEW, R.: Aureomycin and amoebic dysentery. *Lancet.*, **2:** 10, 1950.

BALAMUTH, W., AND BRENT, M. M.: Proof of the direct action of an antibiotic on *Endamoeba histolytica J. Parasitology*, **35:** 23, 1949.

BORDES, L. A., FARINAUD, M. E., AND PORTE, L.: Therapeutic trials with Aureomycin in amoebiasis. *Presse Medicale*, **58:** 198, 1950.

FULLER, F. W., AND FAUST, E. C.: Aureomycin in the cultivation of *Endamoeba histolytica. Science*, **110:** 509, 1949.

GUTCH, C. F.: Aureomycin in the treatment of amebiasis. *New England J. Med.*, **243:** 185, 1950.

HALAWANI, A., ABDULLAH, A., AND EL KORDV, M. I.: Aureomycin in intestinal amoebiasis. A preliminary report. *J. Egypt. Med. Ass.*, **33:** 701, 1950.

HALL, W. H.: The treatment of chronic amebiasis, *New England Med. J.*, **244:** 495, 1951.

HARGREAVES, W. H.: Chronic amoebic dysentery: new approach to treatment, *Lancet.*, **2:** 68, 1945.

HARRELL, G. T., MEADS, M., LAWSON, R. B., AND LIDE, T. N.: Observations on tuberculous meningitis treated with oral Aureomycin. *J. Clin. Invest.*, **28:** 1050, 1949.

HEWITT, R., WALLACE, W., AND WHITE, E.: The effect of Aureomycin on *Endamoeba histolytica in vitro. Science*, **112:** 144, 1950.

HUGHES, J. D.: Treatment of amebiasis with Aureomycin. Report on thirty-eight cases. *J. A. M. A.*, **142:** 1052, 1950.

JONES, W. R.: Antibiotics in the treatment of amoebiasis. *Nature*: **165:** 694, 1059 (Correspondence).

McVAY, L. V., JR., LAIRD, R. L., AND SPRUNT, D. H.: The treatment of amebiasis with aureomycin. *South. Med. J.*, **43:** 308, 1950.

MOST, H., MILLER, J. W., AND GROSSMAN, E. J.: Treatment of amebiasis with bacitracin and other antibiotics. *Am. J. Trop. Med.*, **30:** 491, 1950.

PHILLIPS, B. P., AND REES, C. W.: The growth of *E. histolytica* with live and heat-treated *Trypanosoma cruzi. Am. J. Trop. Med.*, **30:** 185, 1950.

PHILLIPS, B. P.: Cultivation of *Endamoeba histolytica* with *Trypanosoma cruzi. Science*, **111:** 8, 1950.

TODD, J. W.: Suppurative hepatitis and diaphragmatic paralysis. *Lancet*, **1:** 1210, 1950.

WATT, J. Y. C., AND VAN DER GRIFT, W. B.: Laboratory observations on the actions of aureomycin, Circulin, Polymyxins B, D and E on *Endamoeba histoltytica. J. Lab. Clin Med.*, **36:** 741, 1950.

YELLOW FEVER IN PANAMA: HISTORICAL AND CONTEMPORARY

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Existing records of the pathologic anatomy of yellow fever in Panama begin on 21 October 1904, when the first autopsy was performed at the Board of Health Laboratory in Ancon under the direction of Dr. A. B. Herrick. A year later, on 30 October 1905, Autopsy Number 208 was conducted on the body of Joseph J. Trixler, a 42-year-old American foreman residing in La Boca, the twelfth person to die of yellow fever as recorded in the protocols of that period, and the last ever to succumb to an infection acquired in the Canal Zone or Terminal Cities of Colon and Panama.

In order to present the historical background and contemporary status of yellow fever in Panama in a proper perspective, a division of time into the following periods has been made.

Period I.	(1492–1849)
Period II.	(1850–1880)
Period III.	(1881–1904)
Period IV.	(1904–1905)
Period V.	(1906–1929)
Period VI.	(1930–1947)
Period VII.	(1948–1952)

Periods I and II are purely traditional or historical in character.

Data for Period III are derived chiefly from statistical records of the French Canal Companies (*Canal Interocéanique* of Count Ferdinand de Lesseps), and from the publications of Gorgas (1903, 1907, 1908, 1912).

Period IV represents the epoch of total and permanent eradication of yellow fever from the Canal Zone and Terminal Cities by William Crawford Gorgas. Gross anatomical data from the autopsy protocols of the Board of Health Laboratory provide the evidence for this period.

Period V is the interval of imported cases, ending in 1919, and the interval of quiescence prior to the discovery of sylvan yellow fever in Darien. Evidence for this period also is available in the autopsy protocols of the Board of Health Laboratory, with microscopic slides for all fatal cases occurring in 1915 and 1919.

Period VI covers the interval up to and including the discovery of the existence of sylvan yellow fever in eastern Panama as far west as the Bayano River, approximately 35 airline miles east of the Canal, and to the close of World War II. Data for this period are based entirely on mouse protection tests on human serum specimens.

Period VII is that of the contemporary occurrence of human fatalities from sylvan yellow fever west of the Bayano River. Typical microscopic liver lesions provide the specific objective evidence on which data during this recent outbreak are based.

PERIOD I

Pre-Columbian era to the beginning of construction of the Panama Railroad

1492–1849

Guthrie (1916) quotes the early Spanish historians as stating that the followers of Columbus were decimated by a disease described as being "yellow and saffron as gold," and that thereafter, wherever the Spaniards went, the "jaundiced handmaiden of death" was awaiting their arrival. These early accounts, however, have little more than traditional value, for the first historically authentic outbreak in the New World, according to Carter (1931), did not occur until 1648 in Yucatan. Until 1932 the existence of the sylvan form of yellow fever was not recognized, and the urban and sylvan forms were historically indistinguishable. Re-examination of all source material is now essential before proper historical interpretations can be arrived at.

Carter's analysis of the historical aspects of yellow fever in America and Africa is rich in source material, but his interpretations are based wholly on the concept of the urban form transmitted only by the *Aëdes aegypti* mosquito, and he discounts the validity of all accounts in which this factor cannot be demonstrated. The year following the publication of his book, jungle yellow fever was discovered.

Boyce (1911) believed the disease present in Mexico and the Caribbean region almost continuously from the 16th to the 20th centuries. Gorgas (1903) mentions that records indicate the disease was known to Montezuma before the arrival of the Spaniards. Augustin (1909), who made an exhaustive search of the historical background, although he favors the concept of the early existence of the disease in the Caribbean, admits his confusion as to its origin.

Carter does not agree with Boyce and, like Augustin, favors the concept that the disease was introduced from Africa, but in his review of the records of the Mayas in the lowlands of Yucatan, Campeche, and Guatemala, states that they may indicate an ancient knowledge of the disease, for the reference to "Xekik"—vomiting of blood—recurs constantly in the Maya inscriptions, and the prediction of recurring cycles of the pestilence, even in pre-Columbian times, is perhaps implied therein. If jungle yellow fever did exist in pre-Columbian times in the New World, then it must have occurred in its pure form, for the *Aëdes aegyti* mosquito is considered to have been of Old World origin, introduced by the ships of the conquistadores and early settlers.

From 1520, the year in which the route across the Isthmus was first established, to 1820, when South America became free from Spain, the repeated deaths of high Spanish officials traveling in Panama caused wide comment throughout Europe, giving Panama its bad reputation for yellow fever, malaria, and dysentery (Gorgas 1912). From 1820 to the discovery of gold in California in 1849, the Isthmus was seldom used as a route of travel.

For centuries Central America, Mexico, and the Antilles provided a permanent reservoir from which yellow fever invaded other countries. Wooden sailing ships and the early steam vessels, providing their own breeding ground for *Aëdes aegypti*, carried infected mosquitoes over the trade routes of the Atlantic from

Boston to Montevideo, and from southern England to Nigeria. In ports where the mosquito would not thrive, outbreaks were limited to ships.

However one may interpret the historical evidence, the suspicion remains that yellow fever may already have been endemic in Panama at the time of the earliest newcomers. An accurate evaluation cannot be made, however, until a complete reorientation of this evidence has been accomplished.

PERIOD II

Construction of Panama Railroad and period prior to the operation of the French Canal Company

1850–1880

Very little factual data can be found relative to the causes of death in Panama from 1850 to 1880. No vital statistics were compiled by the New Granadan (Colombian) Government, which had no organized sanitary services, nor were any kept by the Panama Railroad. Prevailing opinions indicate that the chief killing disease among the workmen constructing the Panama Railroad was malignant tertian (*falciparum*) malaria, then known as Chagres fever, and that whatever yellow fever may have visited the Isthmus was largely confined to the ships at anchorage in Aspinwall harbor on the Atlantic side (Colon) and off the islands of Flamenco (Deadman's Island), Perico, and Naos on the Pacific side.

During the construction days (1850–1855) the railroad was driven relentlessly toward the Pacific regardless of all obstacles, with results which, as aptly stated by Guthrie, seem superhuman. Along with this construction, swamps and lowlands were drained and filled in. Thus the malaria situation was slightly improved without deliberate intent to do so. Otis (1867) remarks that during the 13 years of operation of the railroad from 1855–1867, 400,000 passengers were transported without a single case of illness as a consequence of the transit, and that diseases contracted prior to 1855 "were purely malarious".

Of several episodes reported during the years 1850–1880 only one indicates that yellow fever was unequivocally present. In 1858 an outbreak of the disease occurred among the ships at anchorage off the islands on the Pacific side, during which 80 members of the crew of the *S. S. Jamestown* died, and a high mortality prevailed on other ships. As was customary at this time, the ships received no assistance from the shore, but had to fight their own battles with yellow fever. These islands were used by the Pacific Mail Steamship Company. Apparently the responsibility of the Panama Railroad for the health of its passengers ended when they arrived at the Pacific terminal.

At one time 800 Chinese laborers were brought directly from Hongkong, only 200 of whom remained alive after a few weeks on the Isthmus. There is no way to determine from what diseases they died. On another occasion a cargo of Irish laborers from Cork arrived at Aspinwall on the Atlantic side. It is said that "so rapidly did they yield to the malignant effects of the climate that not a single day's labor was obtained from a single one of them," and that the survivors were shipped back to New York "where most of them also died from fever fermenting in their blood." Within six months 1,000 Negroes imported from the west coast of Africa had all died off. In 1853 the Fourth Infantry of the United States Army crossed the Isthmus on its way to California. Since the railroad was at this time only half completed, the regiment had to march from Gorgona to Panama City. From the time they left Colon to the time they reached San Francisco they lost 80 men out of a complement of 810, largely due to cholera. Mortality and morbidity from various causes among newly arrived nonimmunes took such a heavy toll that sailors dreaded anchorage in Aspinwall (Colon) harbor.

There is little doubt that the building of the Panama Railroad witnessed a terrific struggle with disease and death, for the mortality was so great that at times construction had to stop because the laboring force had died or were sick, but yellow fever seems to have been largely a maritime scourge at the time, standing guard at the portals of entry and exit until 1880.

PERIOD III

Construction days of the French Canal Companies

1881–April 1904

In 1881, with a spirit of ignorant cheerfulness in some, and superlative courage in others, the workmen of de Lesseps commenced to build the proposed sea-level canal, a project which terminated within seven years in failure. During the years 1881–1888 inclusive, representing the peak of activity of the First French Canal Company, the official records disclose precisely 5,527 deaths from all causes among employees of the *Canal Interocéanique*, while in Panama City from 1883 to 1890 there were a total of 13,106 deaths from all causes. Of these deaths, 1,006 and 905 respectively were attributed to yellow fever.

Gorgas (1912) estimated, however, that the French actually lost 22,189 laborers by death from 1881 to 1889, and that by 1889 there had been 2,000 deaths from yellow fever in the Caucasian force alone of *La Compagnie Général du Canal Océanique de Panamá*, although this force never exceeded 2,500 at any time, and averaged only 1,600. These estimates differ from the official records, but further emphasize the fact that yellow fever, singularly inconspicuous during the construction of the Panama Railroad, had again established itself on the Isthmus. With reference to the facilities provided by the French for the medical care of their employees, Gorgas comments: "They had a most excellent system of hospitals where the sick were well taken care of, and we have the well kept records of these hospitals. They show that during the construction period of the Old French Company, from 1881–1889, 5,618 employees died in these hospitals. But the French were at this time doing the work by contract, and each contractor was charged a dollar a day for each man he had in the hospital. It will readily be understood, therefore, that if the French contractor was anything like the ordinary contractor, not a very large proportion of the sick would go to the hospital."

In Panama City yellow fever was exceeded as a cause of death by malaria, tuberculosis and dysentery, and among the employees of the French Canal Companies it was also exceeded by malaria. Mortality statistics compiled by Gorgas for the entire period 1881–1904 are shown in detail in Tables 1 and 2.

Except for a small outbreak of six deaths in 1897, employees of the French Canal Companies suffered no fatalities from yellow fever after the year 1890, when the Second French Canal Company operated only to maintain the franchise. In Panama City the majority of deaths from yellow fever also occurred during the peak construction days of the First French Canal Company, although the city remained an endemic focus of the disease until 1905. In 1902 there were 202

TABLE 1

Population and deaths from various diseases among employees of the French Canal Companies, from January 1881 to April 1904

YEAR	DEATHS									
	Average No. Employees	Yellow Fever	Beri-Beri	Tuberculosis	Pneumonia	Malaria	Typhoid	Smallpox	Dysentery	Total Deaths
1881	968	11	0	1	0	19	0	0	1	58
1882	1,998	36	0	2	0	40	1	0	7	125
1883	6,941	26	0	19	80	100	18	0	41	423
1884	17,435	128	0	35	135	384	70	0	92	1,232
1885	15,785	199	0	30	128	324	27	0	78	1,096
1886	15,193	308	0	34	88	201	24	11	56	955
1887	16,873	216	123	43	118	202	25	108	75	1,033
1888	13,993	102	69	51	77	98	21	22	40	605
1889	1,939	15	2	12	0	16	4	0	8	91
1890	913	1	2	4	0	5	1	0	2	30
1891	862	0	1	7	0	8	0	0	4	36
1892	796	0	0	3	0	4	0	0	3	22
1893	717	0	0	2	0	4	0	0	7	21
1894	806	0	1	1	0	1	0	0	3	17
1895	1,225	0	0	2	0	4	0	0	3	30
1896	2,715	0	0	4	0	15	0	0	5	79
1897	3,997	6	34	14	0	23	2	0	15	127
1898	3,396	0	0	12	0	13	0	0	3	73
1899	2,500	0	0	15	0	4	0	0	2	57
1900	2,000	0	0	19	0	5	4	0	4	67
1901	2,000	0	0	5	0	4	0	1	0	36
1903	1,450	0	0	5	0	5	0	2	1	33
1903	940	0	0	1	0	3	0	11	3	33
To Apr. 1904	822	0	0	0	0	0	0	0	2	3
Total		1,048	232	321	626	1,482	196	155	455	6,282

First French Company	1881–1888
Receivership	1889–1894
Second French Company	1895–1904 (May)

Published by Isthmian Canal Commission, Government of the Canal Zone, Health Department, W. C. Gorgas, Colonel, M.C., U.S.A., Chief Sanitary Officer, Government Printing Office, Washington, D. C. 1906.

deaths from yellow fever in the city, and the remainder were scattered from year to year, ranging in incidence from none in 1894–1896 and 1898, to 110 in 1900. Since the population of Panama City rose steadily from 20,052 in 1884 to 24,784 in 1905, and deaths from all causes totaled 30,539 during that period, no doubt only the urban form of yellow fever was endemic on the Isthmus, and the turnover must have been rapid to maintain the population level, for life expectancy was evidently short for many reasons besides the presence of yellow fever. The Isthmus and the terminal cities of Panama and what is now Colon continued to be the "stalking ground of death" for the workmen of the *Canal Interocéanique*, especially for newcomers. Of those who chose to become residents, only the few

TABLE 2

Population and deaths from various diseases in the city of Panama, November 1883 to August 1906

YEAR	DEATHS								
	Population	Yellow Fever	Beri-Beri	Tuberculosis	Malaria	Typhoid	Smallpox	Dysentery	Total Deaths
1883	19,827	8	0	12	35	6	3	21	203
1884	20,052	68	0	96	491	36	2	179	2,011
1885	20,276	174	0	123	687	17	0	150	2,228
1886	20,501	281	0	127	497	20	20	123	2,160
1887	20,726	259	16	124	481	26	194	109	2,522
1888	20,952	82	43	128	448	21	40	87	1,893
1889	21,177	33	18	126	249	5	0	71	1,186
1890	21,403	0	11	117	198	10	1	35	903
1891	21,628	17	9	121	178	8	10	37	930
1892	21,853	1	2	138	132	1	0	41	845
1893	22,079	1	1	140	142	2	2	46	903
1894	22,304	0	1	140	137	4	1	40	883
1895	22,528	0	1	141	138	1	2	35	867
1896	22,752	0	9	168	168	4	3	58	979
1897	22,977	45	31	145	203	4	1	56	1,106
1898	23,201	0	17	142	161	4	0	29	951
1899	23,425	87	31	160	190	4	1	34	1,111
1900	23,650	110	64	182	178	8	1	50	1,322
1901	23,874	5	33	132	144	4	124	49	1,209
1902	24,099	202	59	222	562	2	183	282	2,381
1903	24,323	51	72	196	178	0	9	71	1,363
1904	24,548	8	71	127	162	0	2	50	1,233
1905	24,784	29	126	128	148	5	0	60	1,553
To Aug. 1906	—	0	26	103	109	6	0	103	752
Total		1,461	641	3,238	6,016	198	599	1,748	31,494

Published by Isthmian Canal Commission, Government of the Canal Zone, Health Department, W. C. Gorgas, Colonel, M.C., U.S.A., Chief Sanitary Officer, Government Printing Office, Washington, D. C. 1906.

who could develop immunity survived to become part of the permanent population.

PERIOD IV

Epoch of total and permanent eradication of yellow fever from the Canal Zone and terminal cities

May 1904–1905

William Crawford Gorgas was Chief Sanitary Officer in Havana, Cuba, in 1898. Since most of the Havana population was immune, from having survived the disease, only a few deaths per month were occurring from yellow fever, although the city remained a notorious death trap for visitors. Gorgas and his wife had both survived attacks of the disease at Fort Brown, Texas, in 1882, and were immune. Since they were keenly interested in yellow fever they kept in close touch with the activities of the Army Board, headed by Walter Reed, which, in 1900, proved beyond doubt the correctness of the theory advanced since 1881 by Carlos J. Finlay, that the female stegomyia mosquito (*Aëdes aegypti*) was the vector of the disease. Little was then known about the bionomics of this mosquito, and it was generally believed that it was "blown in by the wind." However, in February, 1901, Gorgas abandoned the futile "clean up" campaign directed at trash and filth, and undertook on his own initiative a systematic effort to eradicate the mosquito in Havana.

Many facts were promptly discovered about this vector, especially that it was actually a "house" mosquito. Although many of Gorgas' colleagues, including Walter Reed, were at first skeptical of the effectiveness of his campaign against the mosquito and its larvae, nevertheless, eight months later (September 1901), the city of Havana was completely rid of yellow fever.

In 1904 Gorgas was sent by the Isthmian Canal Commission to Panama to eradicate yellow fever and take charge of the sanitation of the Panama Canal. Upon his arrival, little evidence of yellow fever could be found, although the stegomyia (*Aëdes aegypti*) mosquito was abundant, for there were very few newcomers in the Zone at that time. Nevertheless, recognizing the potential danger, he began his work.

In spite of the proof already established in Havana that a mosquito that could be eradicated was the vector of urban yellow fever, considerable skepticism yet prevailed in executive circles which held stubbornly to the belief that trash and filth were still the cause, thus making Gorgas' work more difficult. Frustrated by inadequacies in supplies and personnel, he felt that his efforts in 1904 got no results whatever, and during the last four months of the year there were eight deaths from the disease in Panama City, six of which occurred in December. Not until direct action was taken by President Theodore Roosevelt were these obstacles finally removed.

The spring of the year 1905 found him fully prepared, and so was "Yellow Jack," which rose in epidemic proportions to accept his challenge. Connor (1916) listed 127 admissions to Ancon (Gorgas) Hospital in 1905 diagnosed as yellow fever, having a monthly distribution as follows:

January	8	June	41
February	14	July	16
March	8	August	12
April	7	September	6
May	14	October	1

He estimated the mortality as 34 per cent. That there were a large number of mild cases of yellow fever in the community which recovered without hospitalization was recognized.

Twelve of the fatal cases in 1905 were subject to autopsy with gross confirmation of the diagnosis by classical descriptions written in the protocols available in the files of the Board of Health Laboratory, and according to a subsequent statement by Darling, some of them were evidently confirmed by microscopic diagnosis of the liver lesion.

Gorgas' campaign against yellow fever in Panama took about 16 months, the first 12 of which were months of little accomplishment in his opinion, for he himself may be quoted as saying that "during the first year giving orders was of no more use than whistling" (1908). In Havana he already had a machine in operation that could quickly be converted to the work. In Panama he had to build up a new machine that did not function efficiently until the last five or six months. In October 1905, the last fatality from yellow fever ever to be acquired in the Canal Zone or the Terminal Cities of Colon or Panama, the death of Joseph J. Trixler, the La Boca foreman, was recorded. There have been none since then other than imported cases brought in by ships from 1906 to 1919. The sylvan epidemic of 1948–1951 did not involve residents of the Canal Zone or the Terminal Cities.

The measures he employed were simple ones and involved three phases: (a) the prevention of breeding of the mosquito by the destruction if its larvae in a city-wide assault on water barrels, cisterns, dishpans and earthen jars, and the installation of an adequate sewage system, (b) destruction of adult mosquitoes by fumigation of all houses with pyrethrum or sulfur, and (c) screening of the sick.

Two months after the last case developed in Panama he found that some of the stegomyia (*Aëdes aegypti*) mosquitoes were still present, and he then formulàted his concept of a critical density, or transmission level, below which there are not sufficient mosquitoes to transmit the disease although some can still be found, which he termed the "yellow fever point."

The years 1904–1905 represent an epoch in the history of tropical sanitation, the exponent of which is William Crawford Gorgas, as the individual who put to immediate practical use the discoveries of his time with such effectiveness that the "Pest Hole of the World" became a clean and sanitary community.

The period 1900–1905 represents the first great epoch in the conquest and control of a scourge that had taken a toll of countless human lives for many centuries, and as a result of the achievements of this epoch Gorgas predicted that from the works of Laveran, Manson, Ross, Reed, Finlay, Carter and many others, all the tropics would eventually be redeemed, as Panama was redeemed, to make a suitable habitation for the Caucasian race.

PERIOD V

Interval of imported cases and quiescence prior to discovery of sylvan yellow fever in Darien

1906–1929

A review of the Annual Reports of the Health Department of the Panama Canal and of the Proceedings of the Canal Zone Medical Association reveals repeated statements that "the last case developed on the Isthmus in November 1905" (Gorgas 1909), or "there have been no cases originating on the Isthmus since 1905" (Fisher 1920). These comments are still valid if one infers that the Canal Zone and Terminal Cities represent "the Isthmus," and the fatality of 1906, about which epidemiologic data are unavailable, is considered as an imported case.

From 1906 to 1919 the summary of imported cases is as follows:

1906—A fatality in May 1906 is mentioned in the Annual Report of the Department of Health of the Isthmian Canal Commission for that year by Gorgas. There is no record of it in the autopsy protocols of the Board of Health Laboratory, and no significant reference is again made to it until the summary tabulated on page 27 of the Report of the Health Department of the Panama Canal for the year 1918 (McCormack 1919). Little is known about it other than that the patient was an Italian, Cantano Sertafine, who became ill in Colon on 17 May 1906 and died in Colon Hospital on 22 May, according to information acquired by Bates (pers. communication). Gorgas states that he was not an employee of the Isthmian Canal Commission. The epidemiology of this case is uncertain, but he was probably a seaman.

1907—A nonfatal case was admitted to Ancon Hospital from the Quarantine Station in July, with the source of the infection stated as the west coast of Mexico or Central America (reported in the Annual Report of the Department of Sanitation of the Isthmian Canal Commission for the year 1907, and also by Connor).

1908—On March 1, a diagnosis of yellow fever was made on a traveller from Guayaquil, Ecuador, (reported in the Annual Report of the Department of Sanitation of the Isthmian Canal Commission for the year 1908, and also by Guthrie and by Connor). There is no record of a fatality.

1909—Two fatalities are recorded in the autopsy protocols of the Board of Health Laboratory, although no reference to their occurrence is made in the Report of the Department of Sanitation of the Isthmian Canal Commission for the year 1909, since in both cases the autopsies were performed aboard ships by Darling.

The first was an unknown white male from Chile, who died after a seven-day illness on a ship of the Southern Pacific Steamship Company and whose body was examined sometime between 3 May and 7 May 1909, eight hours after death, on the island of Flamenco (Deadman's Island) by Darling, who considered the findings typical in all respects, in spite of the absence of black vomit during his illness. This report comprises Protocol No. 1677.

The second, in Protocol No. 1929, was the case of a British passenger on the *S. S. Mexico* en route from Guayaquil, Ecuador, who left that port in apparent good health on 18 December, although in retrospect, "not quite well" at the time. Nausea and vomiting of dark bloody material developed on the twentieth, and he became quite jaundiced before death on the twenty-second. Autopsy, performed aboard ship and limited to the abdomen, disclosed a liver of normal size but presenting yellow mottling and a golden hue on cut sections, with reddish-yellow areas.

1910—Three cases are reported by Guthrie, one from Cartagena, Colombia, in January, and two from Guayaquil, Ecuador, one in March and one in November. All were detected and held at the Quarantine Station except the Cartagena case. This was the case of W. B. Mason, a 28-year-old Englishman, who arrived in Colon on 6 January 1910, and proceeded to Panama City. Although he began to feel ill on 8 January, he remained at work, going to bed on 16 January. He was admitted to Ancon Hospital on 19 January, where he died on 24 January. Black vomit had developed on 22 January and Dr. W. E. Deeks had noted jaundice. Blood oozed from his nose and gums, and he became restless and delirious prior to his death. Autopsy findings, in Protocol No. 1964, were consistent with yellow fever, and the liver was described as uniformly dull yellow in color with red speckling beneath its dome, and typical histologically.

A second fatality for the year 1910 is represented by Protocol No. 2002, listed as the case of Bernard Lenge, age 52, with no clinical, pathologic, or epidemiologic details given. This autopsy was performed on 8 March or 9 March, and is undoubtedly one of the three cases to which reference is made by Guthrie. Only the case of W. B. Mason is mentioned in the Report of the Department of Sanitation of the Isthmian Canal Commission for the year 1910. Clark (pers. communication) recalls that Darling performed the autopsy on Lenge and that he was never admitted to the hospital. Clark also recalls that Darling stressed the microscopic liver findings as far more important than gross descriptions.

1912—Guthrie mentions three cases occurring during the months of January, March, and July, all of whom contracted the disease in Guayaquil, Ecuador. An autopsy was performed on the only fatal case on 5 March, recorded as Protocol No. 2973. This patient was a steward on the *S. S. Chile* by the name of Folette, a mestizo and native of California. Becoming ill with the grippe or malaria on his ship while in Guayaquil, he was sent to the hospital there for a few days, "where he contracted yellow fever." He was detected in the Quarantine Station in the Canal Zone the day before he died in Santo Tomas Hospital, Panama City. His liver was described as undersized but not box-wood or yellow in color, and it was said that "on section the lobules present a characteristic appearance. The peripheries are yellow, the intermediate zones are reddish purple, while the central zones present minute spots of gray." His mouth was stained with black vomit, and a slight jaundice was noted through his tanned skin. Autopsy was limited to the abdomen.

This was the only case to which reference was made in the Report of the Department of Sanitation of the Isthmian Canal Commission for the year 1912, which was the last official report rendered by Gorgas.

1915—Four cases are listed for this year by Connor and Guthrie, and also by the Chief Health Officer, Charles F. Mason, in the Report of the Department of Health of the Panama Canal for the year 1915.

All originated in Buenaventura, Colombia, with an incidence of two in October and two in December, and all four arrived by the *S. S. Jamaica*. Three died, and their autopsy protocols, Nos. 4393, 4414, and 4460, with microscopic slides, are available in the files of the Board of Health Laboratory. Not only are the clinical summaries and autopsy protocols classical in content and expression, but the liver slides on all three, thirty-five years later, still exhibit the typical microscopic lesion of yellow fever. These autopsies were performed by Herbert C. Clark, who served as pathologist at the Board of Health Laboratory from 1909 to October 1917, prior to his service in Europe during World War I, and after his return to the Laboratory in 1919 until November 1922. If the accuracy of the diagnosis in these three cases, readily confirmed today by examination of the liver slides, is a fair criterion of an era of interpretations based largely on clinical data and gross anatomical findings, then most of the data from that period must be acceptable, even although Clark and his associates, with the remarkable exception of Samuel T. Darling, did not fully accept the microscopic pattern of the liver lesion as pathognomonic of the disease.

Referring to the microscopic liver lesion, Clark (1916) maintained: "The character of the fatty change in the middle and peripheral zones of the liver lobules is considered by some as pathognomonic of yellow fever. It does seem true that this feature is more constant and advanced than in other acute infections, but experience at the laboratory shows that cases sometimes occur in other well-established acute infections that offer this fat change in such a confusing manner that I am sure I would not feel secure in offering a diagnosis of yellow fever on the presence of this one feature . . . none of the features, such as jaundice, blood extravasation, black stomach content, etc., when taken singly are reliable elements . . . since they occur singly and sometimes collectively in other conditions."

Clark, with diagnostic acumen proved to have been 100 per cent accurate, reflected the conservative attitude toward the liver lesion that prevailed with few exceptions until 1928, when Hoffman (1928), discounting Councilman's original reluctance to accept his own classic description of the lesion as pathognomonic (1890), declared the microscopic typicality diagnostic in itself.

During the time Samuel T. Darling, the discoverer of histoplasmosis, served as pathologist at the Board of Health Laboratory from 1905 to 1906, and as Chief of the Laboratory from 1906 to 1915, when the pathologists were George Hoyt Whipple (1907–1908), Francis P. McCarthy (1908–1909), and Herbert C. Clark

(1909–1915), the specificity of the liver lesion was evidently recognized, as the following memorandum with Darling's signature indicates:

Board of Health Laboratory,
Ancon, Canal Zone,
July 22, 1912.

Superintendent,
Ancon Hospital.
Sir:

Complying with your request, I have the honor to state that an examination has been made of tissue from the liver and kidneys of Orella Fioilan, who died in Santo Tomas Hospital July 14, 1912. Sections stained with hematoxylin and eosin show none of the characteristic lesions of yellow fever. Sections stained for fatty degeneration show this to a slight degree, but no more than might be encountered in other severe infections.

All cases of yellow fever diagnosed as such in Ancon Hospital or at the Quarantine Station since 1905, and confirmed at autopsy, have been studied histologically and *in all cases I have found extensive and absolutely typical acidophilic necrosis of the hepatic cells* with fatty degeneration, *an unvarying picture*, but there is not a trace of this lesion in the liver of the case reported here.

Respectfully,
(Signed) Samuel T. Darling
Chief of Laboratory

Clark states that Councilman visited the Canal Zone about 1911, and was by that time almost convinced that the lesion he had described in 1890 was specific for yellow fever, although there "might be some conflicting entities" and he "wanted Darling to check." Darling's letter indicates that he himself had already come to a definite conclusion. Clark also states, however, that when test slides were submitted to Darling to prove the point, Darling, although he missed none of the confirmed yellow fever cases, was not certain about some of the liver slides from other entities, such as blackwater fever, lobar pneumonia, and acute atrophy. Following Darling's departure early in 1915, although the liver lesion was considered important, it was no longer emphasized as the specific lesion. Clark felt, however, that there would be no doubt about it in the typical cases.

1919—Deaths of two persons are recorded in the Report of the Health Department for the year 1919, one of whom was chief engineer of a steamship who contracted the disease in Guayaquil, Ecuador late in January, and died 11 February in Colon Hospital, recorded in Protocol No. 5437, and the other an Australian boy en route home from Nicaragua, recorded in Protocol No. 5583. Clark did not perform either of these autopsies. The case of the chief engineer is doubtful, for microscopic specimens of the liver show nothing at all to suggest yellow fever, although conventional fatty infiltration is moderate, and the slide is still in almost perfect condition. The cause of death was at the time suspected to be Weil's disease, and a liver suspension was inoculated into two guinea pigs which showed no signs of illness "at the end of a week."

The second case, however, that of Kenneth Thomas, a 12-year-old Australian boy born in Matagalpa, Nicaragua, was not only clinically typical, with black vomit and bleeding nose and gums, but is readily confirmed by microscopic examination of the liver section after reposing for thirty-two years in the files.

He was ill only three days, and died on 12 August, having been on the Isthmus only two days.

The case of Kenneth Thomas was the last to be imported into the Canal Zone, and the last known fatality from yellow fever on the Isthmus of Panama until November 1948. From 1919 until 1930 there was an interval of inactivity.

PERIOD VI

Discovery of sylvan yellow fever in Darien and region east of the Bayano River

1930–1947

The second great epoch in yellow fever research began in 1927 with the discovery by Stokes, Bauer and Hudson (1928) that an active virus could be transmitted to rhesus monkeys from infected persons, monkeys or mosquitoes, thus disproving the prevailing concept that *Leptospira icteroides*, described by Noguchi in 1919, was the etiologic agent, and reestablishing the conclusions of the Army Board of 1900 that the etiologic agent was probably a virus. At about the same time Hoffman (1928) openly advocated routine microscopic examination of the liver in all deaths from acute febrile disease of short duration, because of the simplicity of the histologic diagnosis. In 1930, following the return of Rocha Lima (1912) to Brazil, and as a result of discussions by him and N. C. Davis on the specificity of the liver lesion of yellow fever (Soper 1951), viscerotomy services were set up in Brazil and other South American countries, which contributed specifically to the discovery by Soper, Penna, Cardoso, Serafim and Frobisher (1933) in 1932, that the disease was occurring in the Valle do Chanaan, Espirito Santo, Brazil, in the absence of *Aëdes aegypti*, which resulted in the recognition of the sylvan form of yellow fever shortly afterwards. In 1930 Max Theiler (1930) discovered the susceptibility of white mice to the virus and the mouse protection test came into practical use to determine the presence of protective substances in the blood of individuals who had been exposed to and recovered from yellow fever, whether their illnesses had been typical, mild, or inapparent.

All of these discoveries were so rapidly coördinated by the Rockefeller staff that their impact was immediately felt in Panama. Between 1929 and 1932, just prior to the discovery of sylvan yellow fever in Brazil, a number of serums from natives in Darien were collected by personnel of Gorgas Memorial Laboratory, Panama City, and sent to the Rockefeller Foundation for protection tests. Although the total number collected is not recalled by Clark, it is known that there was one positive in a nine year old and another in an eleven year old, and that there were three reported doubtful.

In the Annual Report of the Gorgas Memorial Laboratory for 1937, Carl M. Johnson (1938), in describing his survey work on Chagas' disease, stated that studies conducted in coöperation with the Rockefeller Foundation during the previous two years had established that jungle yellow fever, unrecognized as such, occurred among the natives in the Province of Darien, in eastern Panama. Two hundred and seventy specimens of blood serum were sent to the laboratories of the International Health Division of the Rockefeller Foundation in New

York City for investigation with the mouse protection test. From this series, seven, two of which were from individuals under thirteen years of age, gave definite protection. A similar survey of the natives in the villages of the Middle Chagres River, conducted also by Johnson in 1935–1937, disclosed no positives. These findings led to the surveys of Kumm and Crawford (1943).

Kumm and Crawford collected human serums from many localities in Panama and found ten settlements near the Colombian border with positive tests in children born since 1925. The year 1925 was used as a reference point, since the last known case of human yellow fever in Central America or Mexico occurred in 1924, with isolated outbreaks noted in Vera Cruz (1923), British Honduras, El Salvador and Guatemala (1924). The results of the survey established that sylvan yellow fever existed in eastern Panama as far west as the Bayano River valley, involving both the Atlantic and Pacific coastal settlements, as well as inland river communities.

Surveys in Costa Rica by Sawyer (1942) in 1939–1940, and in Mexico and Guatemala by Bustamante, Kumm and Herrera (1942), had disclosed no positive reactions in human blood except in the older age groups, thus substantiating the conclusions reached by Sawyer, Bauer, and Whitman (1937) that yellow fever was no longer endemic north or west of the Panama Canal. The Army Research Board mentioned by Johnson (1938) had also proven the existence of the sylvan form of the disease on the Atlantic coast in the San Blas region of Panama, having obtained serum from one individual under fifteen years of age which gave a positive protection test. This Board likewise found no cases in the middle of the Isthmus or "above" the level of the Canal Zone (west of the Zone). Surveys on the blood of monkeys or other arboreal mammals had not been made, and the evidence that sylvan yellow fever existed only east of the Bayano River was based on the results of the examination of the human population. This conclusion proved to be epidemiologically correct until November 1948.

Jungle or sylvan yellow fever in the Western Hemisphere is commonly known as "woodcutter's disease," for its tree-hole breeding mosquito vectors dwell mostly in the forest canopy where conditions of light, heat, and humidity are optimal for their habitat, with their peak activity at midday. Natives recognize these mosquitoes as the "blue mosquitoes" that attack them after a tree is felled, since they come down with the upper branches of the fallen trees. It is believed that the arboreal mammals, especially primates (monkeys and marmosets) serve as a part of the reservoir of virus from which the tree-top mosquito fauna becomes infected and again acts as another reservoir until the end of each infected brood. Human infection may be acquired in clearings such as rice, corn, cane, yucca and other milpa plantations bordering the forest, where the "blue mosquitoes" come down in a sloping gradient to ground level for fifty yards or so from the forest edge. Hence it is predominantly a disease of native farmers commonly acquired while working in or enlarging the clearings of their farms and plantations. Infection may also be acquired in road construction through virgin forest or by prolonged stops on highways passing through such wooded country or on river travel, due likewise to the down-gradient of the mosquito fauna by the edge of the forest. In all respects, except in its epidemiology, it is the same disease as urban yellow fever.

During the period of World War II the Panama Mobile Force maintained base camps and outposts throughout the central region of Panama, and operated patrols extending as far east as the Bayano River. A principal mission of this ground force was to keep close contact with the rural population, and had sylvan yellow fever attained epidemic proportions, as it did in the latter part of 1948, that fact would surely have been recognized, for although all troops were vaccinated, the Panamanians at that time were not. It appears, then, that the disease remained east of the Bayano River until 1948.

PERIOD VII

Contemporary outbreak of yellow fever in sylvan form

In November and December 1948, like a "bolt from the blue," jungle yellow fever killed five Panamanian farmers in the Pacora District on the Pacific side of the continental divide between the eastern boundary of the Canal Zone and the Bayano River (Elton, *et al.* 1950; Herrera *et al.* 1949). In August and September, 1949, three more farmers died of the disease in the Buena Vista District still east of the Canal Zone, in the forested farm lands between the Trans-Isthmian Highway and Gatun Lake. The vaccination program was immediately intensified in this area, where 1200 more people were inoculated who had not previously reported for vaccination, and the whole of the Republic was blanketed by vaccinating teams as described by Courtney (1950).

In January 1950 a native farmer from the Chagres District just west of the Canal on the Atlantic (north) side of the Isthmus was admitted to Amador Guerrero Hospital in Colon, where he died of yellow fever. When this fatality occurred, it seemed for the first time reasonable to postulate that a wave of sylvan yellow fever was in progress, headed westward through the Atlantic rain forest, and, since this region is very sparsely settled, the medical staff at the Almirante Hospital of the Chiriqui Land Company (United Fruit Company) in northwestern Panama was alerted to watch for cases of the disease in their labor force. Diplomatic channels to Costa Rica were likewise activated, with the warning that the disease could be expected in that country within the next two years.

On 15 April 1951 a Costa Rican chainman, working on a highway survey in the forest near Almirante, was admitted to the Almirante Hospital, where he died of yellow fever contracted from felling trees.

On 24 April 1951 an intensive vaccination program was begun in Costa Rica in anticipation of the arrival of the wave in the vicinity of Puerto Limón by September. This vaccination campaign was further intensified following the recovery of the virus of yellow fever from a patient admitted to Almirante Hospital early in June 1951, who contracted the infection on a farm in Costa Rica just across the border from the Panamanian settlement of Nievecita. The virus was recovered by Dr. Enid de Rodaniche, virologist at Gorgas Memorial Laboratory, from a serum specimen submitted by Dr. Gustav Engler for a protection test. This patient recovered.

Beginning about the middle of July, 1951, human yellow fever lashed out in northeastern Costa Rica along a 100-mile front, running generally in a northwesterly direction from Puerto Limón, in which five epidemic centers were recognizable before the beginning of its subsidence in mid-September, followed by a few sporadic cases. The complete description of this outbreak is properly the prerogative of the Director of Public Health and the staff of San Juan de Dios Hospital in San José, Costa Rica. However, it can be said that admirable work was done there by Costa Rican physicians, public health workers, and laboratory personnel, in which a wealth of findings have been made on the epidemiology, control, and clinical, pathologic anatomic, clinical pathologic, and therapeutic aspects of the disease, much of which is new or has long been sought to confirm the findings of previous investigators.

At the present time (February, 1952) it has just been confirmed that the wave of sylvan yellow fever has, in some manner, actually crossed the Continental Divide in Costa Rica to the Pacific watershed in the southern part of the Province of Puntarenas and is now moving in an eastward direction back into the Pacific watershed of Western Panama. This break-through was heralded during the early part of October, 1951 by the finding of an unusual number of dead monkeys in the vicinity of Potrero Grande, about 25 miles west of the Costa Rica-Panama boundary on the Pacific side of Costa Rica. Shortly afterwards the serum from a patient convalescing from clinically suspected yellow fever was found to give a positive mouse protection test by Dr. Rodaniche. Although the exact situation was somewhat obscure at that time, an intensive revaccination program was undertaken in southwestern Panama by the Ministry of Public Health. No further information was available until notification, by the Director of Public Health of Costa Rica, of the death, on 15 January 1952, of a workman from a mining camp close to the frontier of Panama in the Coto area of Puntarenas. This fatality was proven to be due to yellow fever by histologic examination of the liver at San Juan de Dios Hospital in San José. A month later autopsy tissues were received at Gorgas Memorial Laboratory from a fatality occurring on 10 February 1952, at the United Fruit Company Hospital, Puerto Armuelles, Republic of Panama, on which a positive diagnosis of yellow fever has been made. This patient had been a laborer, not a United Fruit Company employee, at the lighthouse at the tip of the forested Burica Hills Peninsula which juts southward into the Pacific at the boundary line between Costa Rica and Panama. He had not been vaccinated. It appears that jungle yellow fever has again entered the Republic of Panama, this time from Costa Rica on the west, but moving eastward on the Pacific side. How far it will progress remains conjectural, but the Pacific watershed of Panama is largely a relatively arid region, although there are stretches of forest that may permit the wave to advance during the coming rainy season.

So far as can be determined, the wave is travelling very slowly compared with those that have occurred in the past in Brazil, at an over-all velocity of only about 12 to 15 miles per month. However, its behavior in specific localities seems to be conditioned by terrain and seasonal factors. Its periods of quiescence between outbreaks of epizoötic or epidemic episodes are not wholly understood.

The majority of human cases in Panama and Costa Rica have occurred below the 1,000 foot contour, with a few between 1,000 and 1,500 feet elevation, and in a few others the altitude data are not known. There is some evidence that monkeys flee to the highlands if possible when the epizoötic phase strikes their feeding areas. That wild howler monkeys die of yellow fever has been confirmed by pathological examinations of the liver of dead or sick animals made both at San Juan de Dios Hospital in Costa Rica and at the Board of Health Laboratory in the Canal Zone, on specimens collected in Costa Rica by the Director of Public Health.

Epidemiologic experience indicates that the Costa Rican phase may now be

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FIG. 1

1. The Bayano River represents the western limit of enzoötic yellow fever until the last quarter of 1948.

2. The Pacora District is the locale of five deaths from yellow fever in Panamanian farmers in November and December 1948.

3. The Buena Vista District is the site of three deaths from yellow fever in August and September 1949.

4. Barro Colorado Island is the home of about thirty clans of howling monkeys. Studies conducted by Dr. Nicholas E. Collias and Charles Southwick indicate that their numbers were markedly reduced by some epizoötic shortly after the dry season of 1949.

5. A fatality in the Chagres District in 1950 indicated that a wave was moving westward, probably through the sparsely settled Atlantic rain forest of Panama towards Costa Rica. In March 1950 the Costa Rican Minister of Health was notified through diplomatic channels that Costa Rica would be involved in approximately 14–18 months. The Staff of the Almirante Hospital of the Chiriqui Land Company in Western Panama was also asked to be on the alert for the arrival of the epidemic.

6. In April 1951 a fatality from yellow fever occurred at the Almirante Hospital. Within a week an intensive vaccination campaign was undertaken in Costa Rica.

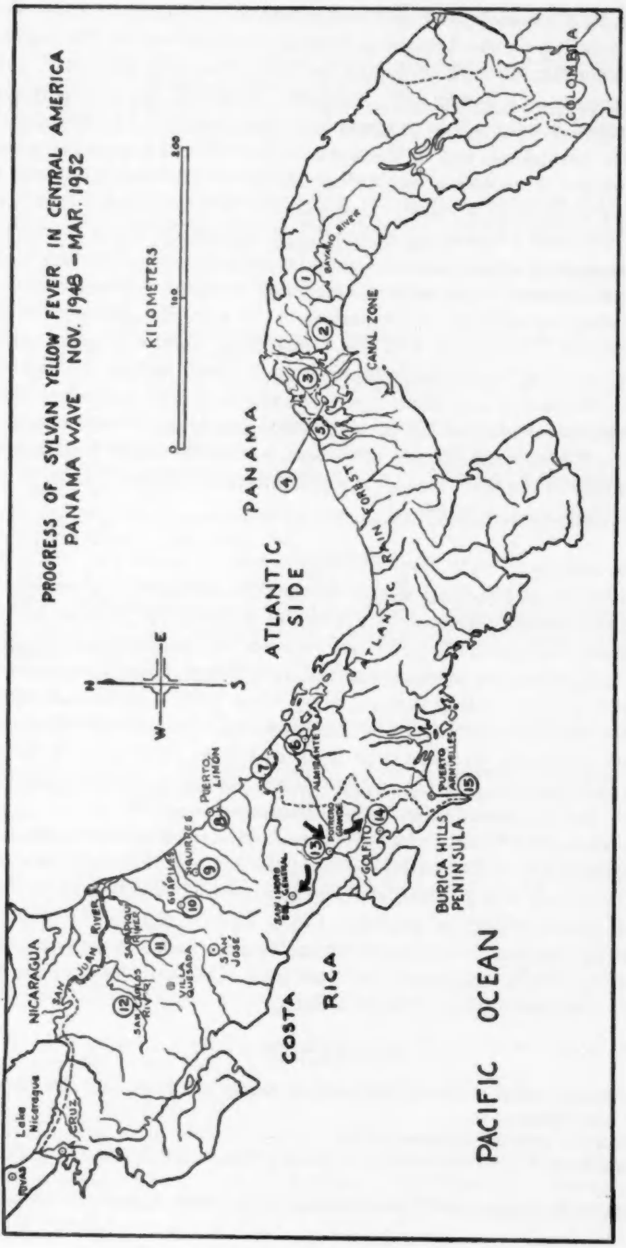
7. In June 1951 the yellow fever virus was recovered from a patient at the Almirante Hospital. This patient had contracted the infection at the Costa Rican Farm of the Chiriqui Land Company across the border from Nievecita, Panama, and recovered.

8-9-10-11-12. These five epidemic centers flared in northern Costa Rica, beginning with a fatality near Puerto Limón (8) on 24 July 1951. Deaths continued until October in the San Carlos watershed (12). Complete autopsies on all fatal cases were performed by Dr. Rodolfo Céspedes Fonseca at San Juan de Dios Hospital, San José. Thirty-three of them have been confirmed pathologically by several laboratories.

13. This is the region of the Cabagra River watershed and the site of a break-through of the wave into the Pacific forest. How this most unforeseen event occurred, involving crossing a minimum elevation of 5,000 feet, is difficult to explain unless the virus was introduced by a human carrier, a migrant farmer, who crossed the divide using a much travelled trail located here, sometime in May or June 1951. The first evidence of involvement of the Pacific watershed was the death of many howling monkeys, reported early in October 1951, in the vicinity of Potrero Grande. This secondary wave is now expanding southeast to the heretofore uninvolved Pacific side of Panama west of the Canal Zone, and northwest up the Pacific Coast of Costa Rica. Only sporadic cases are occurring.

14. The death of a mine laborer from the Coto District at the Golfito Hospital of the United Fruit Company on 15 January 1952 was reported to the Minister of Health of Panama by the Director of Public Health of Costa Rica.

15. A farmer from the Burica Hills Peninsula died in the Puerto Armuelles Hospital of the United Fruit Company in Western Panama on 12 February 1952.


PROGRESS OF SYLVAN YELLOW FEVER IN CENTRAL AMERICA
PANAMA WAVE NOV. 1948 – MAR. 1952
KILOMETERS
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N
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S
W
ATLANTIC SIDE
PANAMA
ATLANTIC RAIN FOREST
CANAL ZONE
BAYANO RIVER
COLOMBIA
NICARAGUA
Lake Nicaragua
RIVAS
STA. CRUZ
SAN JUAN RIVER
SARAPIQUI RIVER
SAN CARLOS RIVER
VILLA QUESADA
SAN JOSÉ
GUÁPILES
SIQUIRRES
PUERTO LIMÓN
ALMIRANTE
POTRERO GRANDE
SAN ISIDRO DEL GENERAL
GOLFITO
PUERTO ARMUELLES
BURICA HILLS PENINSULA
COSTA RICA
PACIFIC OCEAN
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FIG. 1

approaching its conclusion, and that in Panama the disease is already no longer endemic or enzoötic on the Atlantic side of the Isthmus west of the Canal Zone. Definitive experimental work, including the use of "sentinel monkeys," to establish this inference as a fact is still necessary. What will happen in Nicaragua, where an intensive vaccination program is in operation and 102,000 people have already been vaccinated, and in the western countries of Central America and Mexico, promises to become a fascinating chapter in the story of sylvan yellow fever in modern times. It is unlikely that the wave will be arrested spontaneously in its northwestward progression, although not impossible. Many more surprises may be in store, and much still remains to be learned during the next few years. New semi-permanent or permanent stabilized enzoötic centers may become established along its pathway. If the pattern of the wave changes as it has already done in southern Costa Rica, and the Continental Divide is again crossed in other countries, or if it persists in its activity in some regions without moving onward as in the past, the problem it presents will be greatly magnified. Scientific studies should be conducted by entomologists, biologists, veterinarians, mammalogists, virologists, pathologists, clinicians, and public health personnel in this great laboratory of the field, for this is a phenomenon without parallel in Central America perhaps since antiquity.

SUMMARY

A reorientation of historical source material is necessary before conclusions can be drawn concerning the antiquity of sylvan yellow fever. During the building of the Panama Railroad (1850–1855) yellow fever was singularly absent from the mainland, but during the operation of the first French Canal Company (1881–1889) it took a heavy toll of human lives. After 1889 it remained endemic in Panama City until its eradication by Gorgas in 1905. From 1906–1919 only imported cases developed in the Canal Zone and from 1920 to 1948 no further cases were encountered. Surveys of the human population indicate that sylvan yellow fever has been enzoötic in eastern Panama since 1929.

In November and December 1948 a wave of sylvan yellow fever began moving westward from this enzoötic center, following the Atlantic rain forest, and reached northern Costa Rica in July 1951. Early in October 1951 an offshoot of this wave had crossed the Cordillera of southern Costa Rica to the Pacific side and has moved eastward again, re-entering western Panama, but now on the heretofore uninvolved Pacific watershed, west of the Canal. The main front of the wave is expected to continue moving into Nicaragua.

REFERENCES

Augustin, George 1909, *History of Yellow Fever*, Searcy and Pfaff, Ltd., 724-728 Perdido Street, New Orleans, La.

Bates, Lewis B., personal communication.

Boyce, Sir Rubert W. 1911, *Yellow Fever and its Prevention*, E. P. Dutton and Co., New York.

Bustamante, M. E., Kumm, H. W., and Herrera, J. R. 1942, Ausencia de fiebre amarilla en el valle del Usumacinta (Guatemala y Mexico), *Rev. d. Inst. Salub. y Enferm. Trop.*, Mexico, D. F. **3**: 255–271.

Carter, Henry Rose 1931, *Yellow Fever. An Epidemiological and Historical Study of its Place of Origin*, Williams and Wilkins Co., Baltimore.

Clark, Herbert C. 1916, A pathological report on recent cases of yellow fever in Panama, including a differential diagnosis of other diseases showing jaundice, *Proc. Med. Assoc. Isthm. C. Z.* **9**: 80–88.

Clark, Herbert C., personal communication.

Connor, Roland C. 1916, Clinical diagnosis of yellow fever, with notes of symptoms presented by four cases of the disease treated in Ancon Hospital during October and December, 1915, *Proc. Med. Assoc. Isthm. C. Z.* **9**: 65–79.

Councilman, W. T. 1890, *Report on the etiology and prevention of yellow fever*, by George M. Sternberg, pp. 151–159. U. S. Marine Hospital Service, Government Printing Office, Washirgton, D. C.

Courtney, K. O. 1950, Report on the recent outbreak of jungle yellow fever in Panama, *Am. J. Pub. Health* **40**: 417–426.

Elton, N. W., and Johnson, C. M. 1950, Pathologic features of yellow fever in Panama, *U. S. Armed Forces Med. J.* **1**: 596–601.

Fisher, H. C., Chief Health Officer 1920, *Report of the Health Department of the Panama Canal for the Calendar Year 1919*, The Panama Canal Press, Mt. Hope, C. Z., p. 23.

Gorgas, W. C. 1903, Disappearance of yellow fever from Havana, Cuba, *Med. News*, New York, January 3, 1903.

Gorgas, W. C. 1907, Sanitary work on the Isthmus of Panama during the last three years, *Med. Record*, May 18, pp. 1–15.

Gorgas, W. C. 1908, Method of spread of yellow fever, *Proc. Canal Zone Med. Assoc., 1908*, Press of the Am. Med. Assoc., Chicago, Ill.

Gorgas, W. C., Chief Health Officer, 1909, Annual Report of the Department of Sanitation of the Isthmian Canal Commission for the year 1908, Government Printing Office, Washington, D. C., p. 7.

Gorgas, W. C. 1912, Sanitation at Panama, *Journal of the American Medical Association* **58**: 907–909.

Guthrie, Marshall C. 1916, The Isthmus and yellow fever, *Proc. Med. Assoc. Isthm. C. Z.*, **9**: 53–64.

Herrera, J. M., Elton, N. W., and Nicosia, J. 1949, La aparicion en Panamá de un brote de fiebre amarilla en su forma "selvatica" (1948–1949) descubierto por el examen postmortem, *Archivos del Hospital Santo Tomas* **4**: 1–49, (July–December).

Hoffmann, W. H. 1928, Diagnosis of endemic yellow fever, *Am. J. of Trop. Med.* **8**: 563–568.

Johnson, Carl M. 1938, *Annual Report of the Gorgas Memorial Laboratory*, 1937, 75th Congress, 3d Session, House Document No. 467, U. S. Govt. Printing Off., Washington, D. C., p. 8.

Kumm, H. W., and Crawford, P. J. 1943, Recent distribution of endemic yellow fever in Central America and neighboring countries, *Am. J. Trop. Med.* **23**: 421–431.

McCormack, Arthur T., Chief Health Officer, 1919, *Report of the Health Department of the Panama Canal for the Calendar Year 1918*, The Panama Canal Press, Mt. Hope, C. Z., p. 27.

Otis, F. N. 1867, *History of the Panama Railroad and Pacific Mail Steamship Company*, Harper and Bros., New York.

Rocha Lima, H. da 1912, Zur pathologischen Anatomie des Gelbfiebers, *Verhandl. d. Deutsch. path. Gesellsch.* **15**: 163–181.

Sawyer, W. A. 1942, La fiebre amarilla en las Americas, *Bol. Ofic. San. Panam.* **21**: 320–333.

Sawyer, W. A., Bauer, J. H., and Whitman, L. 1937, Distribution of yellow fever immunity in North America, Central America and the West Indies, Europe, Asia, and Australia, with special reference to the specificity of the protection test, *Am. J. Trop. Med.* **17**: 137–161.

Soper, F. L. 1951, personal communication.

Soper, F. L., Penna, H., Cardoso, E., Serafim, J., Frobisher, M., and Pinheiro, J. 1933, Yellow fever without *Aedes aegypti*. Study of a rural epidemic in the Valle do Chanaan, Espirito Santo, Brazil, *Am. J. Hyg.* **18:** 555–587.

Stokes, A., Bauer, J. H., and Hudson, N. P. 1928, Experimental transmission of yellow fever to laboratory animals, *Am. J. Trop. Med.* **8:** 103–164.

Theiler, Max 1930, Studies on the action of yellow fever virus in mice, *Am. Trop. Med. and Parasitol.* **24:** 249–272.

LEPTOSPIROSIS IN NORTH AMERICA

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Recognition of leptospirosis in man and animals in North America is increasing. Recent studies show these disease entities to be of far greater significance than has been attributed to them in the past.

For many years leptospirosis and Weil's disease were considered synonymous. Spirochaetal jaundice was the only clinically recognized leptospirosis. The rat borne classical *L. icterohemorrhagiae* was the only recognized strain. Meyer and his associates (1938) reported the first human cases of canicola fever in the United States, and found the dog to be the reservoir of this infection. This marked the beginning of intensive study of the leptospiroses in North America, and has resulted in increasing awareness of the importance of these infections in man and in the domestic animals.

MORPHOLOGY

Leptospirae are long, extremely thin organisms, varying from five to forty microns in length and approximately 0.1 microns in width. They are highly motile, and are capable of passing through the pores of Seitz E-K filter pads. In fluid mediums, characteristic hooks are seen at both ends of the organism, and movement is by a spinning motion on the longitudinal axis. In semisolid mediums, the organism shows a characteristic boring movement. Unpublished electron microscopy studies in our laboratory show the organism to possess a spiral flagellum coiled along its entire length. This flagellum is of higher electron density than the central filament and one of its components is presumably a nucleic acid.

SPECIES AND HOST RANGES

The classical *L. icterohemorrhagiae* strain has been found in the rat population throughout the United States and Puerto Rico. Randall and Cooper (1944) isolated it from a naturally infected dog. Isolation and serologic evidence (Gochenour *et al.* 1951) reveal canine infection with *L. icterohemorrhagiae* in Puerto Rico. Up to the present time, *L. canicola* infections appear to be limited to the dog and to man.

Baker and Little (1948) isolated leptospirae from an outbreak of disease in dairy cattle in New Jersey. This strain was studied in our laboratory and found to be antigenically indistinguishable from *L. pomona*. To the present time, *L. pomona* has been isolated from cattle in New Jersey, New York (Reinhard *et al.* 1950), Pennsylvania (Little *et al.* 1950), California (Meyer) and Georgia (Sippel). Serologic evidence (unpublished) shows bovine leptospirosis to be present in nineteen states. The author and his associates (1950), have determined, on the basis of serologic evidence, that *L. pomona* is the etiologic agent of recurrent iridocyclitis of horses. Gochenour and his associates (in press) isolated *L. pomona* from swine in Ohio, the first isolation from hogs in the United States. Beeson *et. al.* (1951) reported the first human case of infection with *L. pomona* in the United States. Schaeffer (1951) reported an outbreak of leptospirosis pomona in a group of young adults who contracted the infection while swimming in a slow moving stream about which cattle and swine were pastured. Finally and important is the fact that *L. pomona* is regarded in Europe as the causal agent of swineherd's disease.

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During investigation of an outbreak of leptospirosis in Puerto Rico, five antigenically distinct strains of leptospirae were isolated from human cases and from the rodent population. These were *L. icterohemorrhagiae*, *L. ballum*, and strains falling in the *L. bataviae*, the *L. pyrogenes* and the *L. hebdomadis* groups. Rats, mice and mongooses were found to harbor leptospirae in this area. Definitive identification of these strains will be the subject of a forthcoming report.

EPIDEMIOLOGY

The reservoir of leptospirae pathogenic for man are rodents and the common domestic animals. These infected hosts excrete the organisms in their urine for an indefinite period of time. They survive for several weeks in water on the alkaline side of neutrality (van Thiel 1948). A single virulent leptospira is sufficient to cause infection in the guinea pig. This may be true of man who is more susceptible to leptospiral infections (van Thiel *et al.* 1941).

Leptospirae may gain access to the body through minute breaks in the skin or through the mucous membranes of the eye, nose, mouth or vagina (van Thiel 1948a). The eye and the nasal mucosa are excellent portals of entry, and in our opinion, a large number of infections occur via these routes. Man may come in direct contact with infected urine, or due to his occupation, may come in contact with water containing pathogenic leptospirae. Food and drinking water contaminated with urine of infected mice or rats is a potential source of infection. From a military viewpoint, the greatest hazard of infection occurs when troops are forced to wade through or are "pinned down" in infected waters.

The incidence of the disease in any locality is dependent on the mean population density of man and on the degree of leptospiral infection in the animal hosts.

CLINICAL MANIFESTATIONS

The leptospiral infections in man may be the characteristic Weil's or swineherds' disease, or they may simulate typhoid fever, influenza, or benign bacterial meningitis. Uveitis and iridocyclitis are common late sequelae of infection. Hall and his associates (1951) have described clinical and pathological findings in their studies on evaluation of antibiotic therapy in leptospirosis. Beeson *et al.* (in press) have reported a series of cases of "pure leptospiral meningitis." In general, the disease is sudden in onset and characterized by a high spiking fever. The patient usually complains of chills, fever, severe frontal headache, and severe pains in the muscles of the legs and back. Burning of the eyes and pronounced scleral injection are characteristic of the onset of the disease. It is important that only approximately one third of the human cases of leptospirosis become icteric.

LABORATORY DIAGNOSIS

The diagnosis of leptospirosis may be confirmed in the laboratory either serologically or bacteriologically. A detailed description of the procedures employed in our laboratory is in preparation (Gochenour *et al.*).

Serological confirmation of the diagnosis of leptospirosis may be made by either complement fixation or by agglutination-lysis methods. Serial serum specimens are collected immediately following onset of the disease and at weekly intervals thereafter. Clinical diagnosis is considered confirmed if a rise in either complement fixing or agglutination-lysis titer is demonstrated. Single serum examinations may be considered of diagnostic significance if an agglutination-lysis titer of 1:400 or greater, or a 4-plus complement fixation reaction with undiluted serum is demonstrated. These two serological tests have been compared on a series of bacteriologically proved cases of leptospirosis and have been found to be equally specific and reliable.

Bacteriological confirmation of clinical diagnoses is afforded by demonstration of leptospirae in the blood, the cerebrospinal fluid or the urine. Leptospirae are found in the blood and cerebrospinal fluid during the initial febrile period of the disease, rarely after the 7th day. They seldom appear in the urine prior to the 10th day of disease.

Direct examination of blood, cerebrospinal fluid, or urine by darkground illumination is hazardous even when interpreted by experienced investigators. Artefacts such as the protoplasmic extrusions from platelets and erythrocytes are always misleading. Diagnosis of leptospirosis therefore should never be made on the findings of direct darkground examination alone.

In our experience, direct culture of venous blood or cerebrospinal fluid is preferable to the inoculation of laboratory animals. Fletcher's (1927) semisolid medium is the medium of choice. It is essential to employ duplicate tubes of medium, and to inoculate with minimal quantities of blood or cerebrospinal fluid: 0.03 ml., —one drop from a 19-gauge needle—is optimal for a tube containing 5 ml. of medium. Direct inoculation at the bedside is preferable but inoculation within four hours has been quite successful with blood defibrinated by shaking with glass beads. Cultures are incubated at 30°C. and examined on the 7th, 14th and 28th days. No culture should be discarded as negative until after at least 28 days' incubation.

On account of bacterial contamination, direct culture of urine is not feasible; animal inoculation must be employed. The patient's urine should be alkalinized by oral administration of sodium bicarbonate during the 24 hours preceding collection of the urine sample. The urine is centrifuged at 17,000 r.p.m. in a refrigerated International centrifuge for thirty minutes. The supernate is discarded and the sediment suspended in a minimal amount of physiological saline solution. This is inoculated intraperitoneally into 25–35 gram hamsters. The animals are bled by cardiac puncture on the fourth day post-inoculation and the heart's blood cultured in Fletcher's medium as before described.

TREATMENT

Hall and associates (1951) critically appraised the use of chloramphenicol, aureomycin, penicillin, streptomycin, combined aureomycin and streptomycin, and cortisone and aureomycin in the treatment of human leptospirosis. They concluded that none of these agents were effective in the therapy of these infections. Brunner and Meyer (1949, 1950) found aureomycin and streptomycin effective in both the therapy of clinical canine leptospirosis and in the elimination of renal infection in known chronic carriers.

DISCUSSION

Experience gained in the past two years indicates that the leptospiroses are more common infections of man and animals in North America than formerly realized. Failure to recognize the disease combined with an inadequate number of leptospiral diagnostic laboratories has resulted in erroneous diagnoses ranging from viral encephalitis to fevers of unknown origin. Evidence at present (unpublished) indicates that ophthalmic sequelae are related to the continuing presence of leptospirae in the anterior chamber fluid. We would therefore postulate that iritis and iridocyclitis occurring as sequelae to apparent or inapparent leptospiral infections are the result of the survival of leptospirae in the anterior chamber fluid. An awareness of the presence of leptospirosis in man and in animals in North America will result in greater knowledge of its prevalence and geographical distribution. Diagnostic facilities at state and federal levels are urgently needed. Epidemiological surveys are needed to determine the prevalence of infection in man and the lower animals. Development of a simple, reliable and rapid field test is especially desirable.

SUMMARY

Leptospirosis in North America may be caused by any of at least seven antigenically distinct strains of leptospirae. Of these, *L. icterohemorrhagiae*, *L. canicola*, *L. pomona* and *L. bataviae* are known to exist in the United States. Methods for serological and bacteriological diagnosis of leptospirosis have been discussed. The need for more adequate diagnostic laboratory services, improved diagnostic procedures, a definitive therapy and epidemiological surveys has been set forth.

REFERENCES

Baker, J. A., and Little, R. B. 1948. Leptospirosis in cattle, *J. Exper. Med.* **88**: 295–308.

Beeson, P. B., and Hankey, D. C. (in press), "Benign aseptic meningitis" as a manifestation of leptospiral infection.

Beeson, P. B., Hankey, D. D., and Cooper, C. F. 1951, Leptospiral iridocyclitis, *J.A.M.A.* **145**: 229–230.

Brunner, K. T., and Meyer, K. F. 1949, Streptomycin in the treatment of leptospira carriers. Experiments with hamsters and dogs, *Proc. Soc. Exper. Biol. & Med.* **70**: 450–452.

Brunner, K. T., **and Meyer**, K. F. 1950, Effect of aureomycin on *L. canicola* and *L. icterohemorrhagiae* in vitro and experimental carrier studies, *Am. Vet. Res.* **11:** 89–90.

Fletcher, W. (1927-1928), Recent work on leptospirosis, tsutsugamushi disease, and tropical typhus in the Federated Malay States, *Trans. Roy. Soc. Trop. Med. & Hyg.* **21:** 265–282.

Gochenour, W. S., **Jr.**, **Johnston**, R. V., **Yager**, R. H., **and Gochenour**, W. S. (in press), Porcine leptospirosis, *Am. J. Vet. Res.*

Gochenour, W. S., **Yager**, R. H., **Smadel**, J. E., **Wetmore**, P. W., **and Hightower**, J. A. (in preparation), Laboratory diagnosis of leptospirosis.

Gochenour, W. S., **Jr.**, **Yager**, R. H., **Wetmore**, P. W., **Evans**, L. B., **Byrne**, R. J., **Alexander**, A., **and Hightower**, J. 1951, Indonesian Weil's disease in Puerto Rico and the United States, *Fed. Proc.* **10:** 408–409.

Gsell, O. 1946, Leptospirosis pomona, die schweinhuterkrankheit, *Schweiz. Med. Wchnschr.* **76:** 237–241.

Hall, H. E., **Hightower**, J. A., **Pons**, E., **Byrne**, R. J., **Smadel**, J. E., **and Woodward**, T. E. 1951, Evaluation of antibiotic therapy in human leptospirosis, *Ann. Int. Med.* **35:** 981–998.

Little, R. B., **Beck**, J. D., **and McCahon**, J. V. 1950, An outbreak of bovine leptospirosis in Pennsylvania, *Vet. Med.* **45:** 104–110.

Meyer, K. F. Personal communication.

Meyer, K. F., **Stewart**, **Stewart-Anderson**, B., **and Eddie**, B. 1938, Canicola fever, a professional hazard, *J.A.V.M.A.* **93:** 332–333.

Randall, R., **and Cooper**, H. K. 1944, The golden hamster (*Cricetus auratus*) as a test animal for the diagnosis of leptospirosis, *Science* **100:** 133–134.

Reinhard, K. R., **Tierney**, W. F., **and Roberts**, S. J. 1950, A study of two enzootic occurrences of bovine leptospirosis, *Cornell Vet.* **40:** 148–164.

Schaeffer, M. 1951, Leptospiral meningitis. Investigation of a water-borne epidemic due to *L. pomona*, *Proc. 43d Ann. Meet. Am. Soc. Clin. Invest.*

Sippel, W. L. Personal communication.

Unpublished data from our laboratory.

Van Thiel, P. H. 1948, The Leptospiroses. Univ. Pers Leiden, pp. 56, 60.

Van Thiel, P. H. 1948 a, The Leptospiroses. Univ. Pers Leiden, p. 67.

Van Thiel, P. H., **and Veer**, W. L. C. 1941, Biologische Methoden zum Isolieren von *L. icterohaemorrhagiae* aus Wasser, *Acta Brev. Neerl.* **11:** 21–24.

Yager, R. H., **Gochenour**, W. S., **Jr.**, **and Wetmore**, P. W. 1950, Recurrent iridocyclitis (periodic opthalmia) of horses, *J.A.V.M.A.* **117:** 207–209.

HISTOPLASMOSIS ON THE ISTHMUS OF PANAMA

Summary of Epidemiologic Survey (1949–1950) and Report of Fourth Local Case (1951)

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For nearly 45 years one of the enigmas of tropical medicine was the status of *Histoplasma capsulatum* on the Isthmus of Panama. In December, 1905, Dr. Samuel Taylor Darling (1906) encountered at autopsy at the Board of Health Laboratory, Ancon, the first case of histoplasmosis. Within ten months he found two more victims of the disease (1908) and then, in spite of continuous search, the fungus was not seen again locally until a canine infection was recognized by Tomlinson and Grocott (1945). Although it seemed likely the dog contracted its infection in this area, Darling's human cases were immigrants and there was justifiable doubt as to whether the fungus had become established in this region.

During recent years this problem intrigued several workers who tested comparatively small series of patients with histoplasmin at Gorgas Hospital (Wedding *et al.*, 1950) and in Panama City (Mastellari), but these data have not been published. The epidemiologic survey to be summarized here represents the first investigation from the Isthmus of Panama designed to study a large segment of the local population by means of intradermal testing, chest roentgenography, and serologic, cultural and pathologic procedures as indicated. In no case was *Histoplasma* isolated but the results obtained seemed to justify the deductions that the fungus was probably endemic here and that additional search would disclose human infections (Tucker, in press). These deductions were borne out in March, 1951, when the fourth local case of histoplasmosis was recognized, the first in nearly 45 years. This was, moreover, the first unquestionably autochthonous infection from the Isthmus and will be discussed in the following text.

EPIDEMIOLOGIC SURVEY: METHOD AND RESULTS

Between October, 1949 and July, 1950, 1,000 patients were studied on the wards of Colon Hospital, Cristobal, Canal Zone. Pertinent details concerning the general sample and individual entries have been given elsewhere (Tucker, 1951). The patients studied ranged in age from less than one day to 85 years and were representative of the indigenous Isthmian population. Each subject, in addition to interview and routine physical and laboratory examinations, was tested intradermally with 0.1 cc. of one or both histoplasmins used (1:100), one or both coccidioidins used (1:100), and with tuberculin (0.0005 mgm. PPD). In an effort to determine whether cross reactions occurred with these or other commonly employed fungous antigens, 199 patients selected at random within the sample were also tested with candidin (1:10^9) and 241 others with blastomycin (1:1,000). Induration at the site of injection five or more mm. in diameter was considered a positive reaction. All tests were performed and interpreted by the writer between 40 and 48 hours later. A 14 by 17 inch roentgenogram of the chest was obtained on each patient and these were examined by me at the end of the study for calcific pulmonary foci, without reference to the intradermal test results.

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In patients showing intradermal sensitivity to histoplasmin or clinical signs suggestive of histoplasmosis, supplementary diagnostic procedures were carried out whenever possible. Sera were examined for specific humoral antibodies, microscopic and cultural studies were done on blood, sputum, gastric secretions, exudates, tissues taken for biopsy and necropsy material.

TABLE I

Prevalence of histoplasmin and tuberculin sensitivities among 1,000 patients, by ten-year age groups

TEN-YEAR AGE GROUP	TOTAL HISTOPL +		HISTO + TBLIN 0		HISTO + TBLIN +		HISTO 0 TBLIN +		NON-REACTORS		TOTAL PATIENTS TESTED
	No.	P R.*	No.	P.R.	No.	P.R.	No.	P.R.	No.	P.R.	
0–9	4	1.7	4	1.7	—	—	22	9.6	204	88.7	230
10–19	20	35.1	12	21.1	8	14.0	17	29.8	20	35.1	57
20–29	108	54.5	29	14.6	79	39.9	66	33.4	24	12.1	198
30–39	110	54.8	14	7.0	96	47.8	78	38.8	13	6.4	201
40–49	47	51.1	5	5.4	42	45.7	36	39.2	9	9.7	92
50–59	51	55.4	7	7.6	44	47.8	31	33.7	10	10.9	92
60–69	40	38.4	4	3.8	36	34.6	43	41.3	21	20.3	104
70–79	7	30.4	2	8.7	5	21.7	8	34.8	8	34.8	23
80–89	—	—	—	—	—	—	2	66.7	1	33.3	3
All Ages..........	387	38.7	77	7.7	310	31.0	303	30.3	310	31.0	1,000

* Each prevalence ratio (P.R. = percentage of total tested in age group) in this column represents the sum of ratios in next two columns.

Results of this work were of interest, although in no case was *Histoplasma* isolated. The prevalence ratios to histoplasmin and tuberculin by age groups have been summarized in Table I. Of the 387 who reacted to histoplasmin, 77 patients were sensitive to this antigen only, the remainder being tuberculin-positive as well. As I have pointed out elsewhere, the parallel series of coccidioidin tests provided a reliable control (Tucker, 1951). Analysis of results obtained with the heterologous fungous antigens (e. g., candidin, blastomycin, coccidioidin) suggested that whereas each might well have been associated with some few cross reactions, it was highly improbable that these could account for prevalence ratios of the magnitude shown in Table I.

One or more pulmonary calcific lesions were seen in roentgenograms of 113 patients. Among the 77 who reacted to histoplasmin alone, such foci were seen in 15.6 per cent, approximately the same as in patients sensitive to both histoplasmin and tuberculin (17.4 per cent) and to tuberculin only (10.9 per cent), and over three times as great a percentage as among those reacting to neither antigen (4.5). Even though foci in patients reacting only to histoplasmin could not be considered conclusive evidence of infection with the fungus since "pulmonary calcifications are apparently nonspecific reactions to a variety of organisms" (Bass, 1950), it has been well shown by Furcolow (1949) and by Beadenkopf *et al.* (1949) that *Histoplasma* may be the causative agent in many instances. These data were strikingly similar to those reported from histoplasmosis-endemic centers, not only in the United States, but from Latin America as well, and by analogy supported the thesis that *Histoplasma* probably occurred here.

The supplementary diagnostic studies did not further clarify the situation, an anticipated result. It was known that Pará (1946) had found only five cases of histoplasmosis by the examination of 169,808 hepatic specimens obtained by viscerotomy in Brazil, a ratio of 1:34,000. As of January 1950, only 138 authenticated cases had been recognized throughout the world (Hodgson *et al.* 1951). None of the 226 separate tests for humoral antibodies on sera from 166 reactors showed significant titers of complement-fixing or collodion particle-agglutinating antibodies (Salvin, 1947, Campbell *et al.*, 1949). Microscropic studies in the total group of 387 reactors to histoplasmin numbered 795, and there were 167 cultural procedures carried out. *H. capsulatum* was not found.

In drawing final conclusions from this investigation, therefore, the failure to isolate the fungus in the comparatively small series of patients studied was given little weight. On the other hand, the remarkable prevalence of intradermal sensitivity to histoplasmin, the high prevalence of calcific pulmonary foci among these reactors, and the striking similarity of these findings to those reported by investigators in known histoplasmosis-endemic regions, appeared to justify the following statement: "The sum of the circumstantial evidence would support the contention that histoplasmosis does occur on the Isthmus of Panama, apparently in the non-fatal or 'benign' form. These data do not in themselves prove the existence of *H. capsulatum* here but suggest that further search may disclose additional local cases in the future" (Tucker, in press).

REPORT OF CASE AND CONTACT INVESTIGATION

On February 7, 1951 a three month old Panamanian infant was admitted to Gorgas Hospital (Chart #648,927), on the service of Dr. J. R. Mitchell, because of failure to gain weight properly, fever and abdominal enlargement. The baby had been born in this hospital on November 10, 1950, about one month before the expected date of delivery, weighing five pounds, four ounces (2,380 grams). Although delivered by breech extraction, the infant breathed and cried spontaneously. At the age of one week he was taken home against advice. According to the mother, the child did not do well from the start. Appetite was poor, and within a few days it was apparent that the child was sickly. Intermittent fever was noted and there was progressive abdominal enlargement.

At the time of admission to the ward at the age of 90 days, the obviously ill male child weighed only seven pounds, eight ounces (3,400 grams). The striking features on physical examination were marked pallor and hepatosplenomegaly. There were clinical and roentgenologic signs of broncho-pneumonia (Fig. 1). Laboratory studies showed depression of all formed elements in the circulating blood (hypersplenism?) but a marrow smear was described as unsatisfactory because of admixture with peripheral blood. In spite of all efforts the baby went progressively downhill and died on March 16th. Clinical diagnoses were: bronchopneumonia, aplastic anemia and hepatosplenomegaly, causes undetermined.

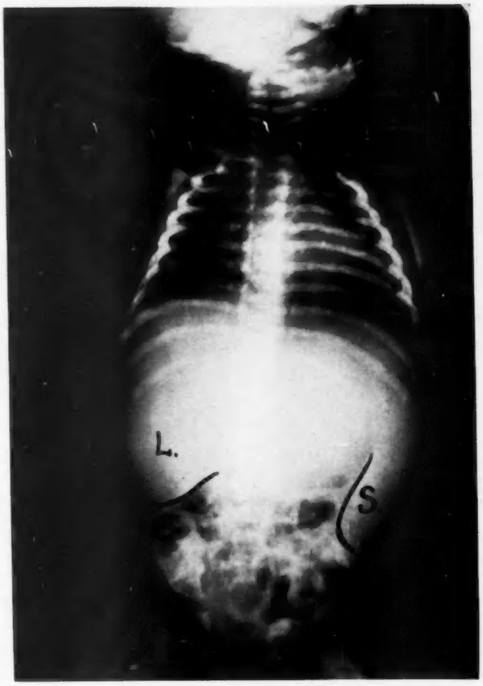

FIG. 1. Roentgenogram of infant with histoplasmosis. The perihilar infiltration in the right hemithorax was more apparent in the original film than in the photograph, but the tremendous enlargement of the liver and spleen is well shown.

At necropsy (Dr. J. R. Draheim) the gross appearance suggested tuberculosis, but microscopic studies of tissue sections and smears by Drs. Joel Shrager, C. M. Johnson, N. R. Elton and other members of the Board of Health Laboratory Staff showed that the cause of death had been generalized histoplasmosis. This morphologic diagnosis was later confirmed by Drs. J. F. Kessel, M. Biddle and other mycologists at the Los Angeles County General Hospital, California. Cultures were overgrown with *Candida albicans*. Further clinicopathologic details will be published elsewhere by the necropsy surgeon.

Contact investigation was started at once. The mother was 40 years of age and had never been away from the Isthmus. She had been pregnant twice before, the first ending in a stillbirth at eight months, the second, in August, 1949, yielding a healthy daughter. Except for roseola as a child she had never been ill and chest roentgenograms taken during the last two pregnancies were described as normal. The father was born in Jamaica, British West Indies, and had immigrated to the Isthmus as a young man. His past personal history was not remarkable. There was no evidence of chronic or recent acute illness in the maternal grandparents who occupied quarters with the parents.

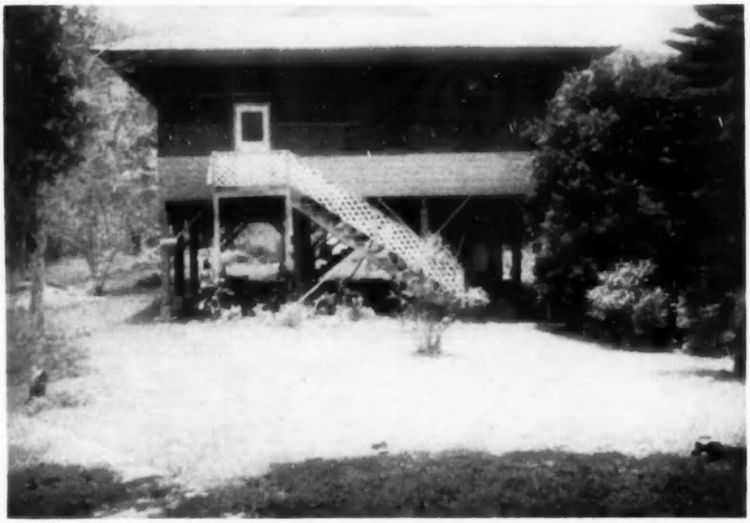

FIG. 2. House and environs in which patient with histoplasmosis lived from the age of one week until final hospital admission on February 7, 1951, at the age of 90 days. It was the impression of the family that the baby had become ill during the first month of life. At the time of admission to the ward the disease must have been widely disseminated and far advanced.

The family lived in the elevated frame house of unusually good construction shown in Fig. 2. Located in the outlying portion of the small Panamanian village of Chilibre, the home was near the Canal Zone-Republic of Panamá border and roughly ten miles from the Miraflores locks where Darling's Chinese patient, Fu Mas, had kept a shop for about 15 years prior to his death from histoplasmosis in August, 1906. Approximately 100 meters east of the paved trans-Isthmian highway to the Madden Dam, the home was clean and tightly screened. Typical tropical vegetation came up to two edges of the yard. All family members and pets were tested intradermally with appropriate antigens and sera were obtained for tests for humoral antibodies, with the results shown in Table II. It was not technically feasible to test the many chickens which ran about freely

TABLE II

Summary of results of contact investigation of fourth case of histoplasmosis on the Isthmus of Panamá

SUBJECTS STUDIED	AGE IN YEARS	PULMONARY CALCIFIC FOCI	INTRADERMAL SENSITIVITY			TESTS FOR HUMORAL ANTIBODIES				REMARKS
			Histopl	Tblin	Coccid.	Histo C.F.[1]	Histo C.A.[2]	Blastom.	Coccid.	
Reported case	4/12	None								*Histoplasma* demonstrated in tissues
Mother	40	None	2+ (11 mm[3])	2 + 13 mm.	Neg.	Neg.	Neg.	Neg.	Neg.	
Father	40	Several small foci	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	
Sister	1½	None	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	*Histoplasma* not found
Maternal grandmother	68	None	1+ (5 mm.)	2 + 11 mm.	Neg.	Neg.	Neg.	Neg.	Neg.	
Maternal grandfather	70	Several small foci	4+ (12 mm.) with necrosis	2 + 11 mm.	Neg.	1:5[4]	Neg.	Neg.	Neg.	
Dog #1	?		Neg.			A.C.[5]	Neg.	A.C.	A.C.	Clinically well
						A.C.	1+[4]	A.C.	A.C.	
Dog #2	?		1+ (7 mm.)			A.C.	Neg.	A.C.	A.C.	Clinically well
						A.C.	1+	A.C.	A.C.	
Cat	?		Neg.			Neg.	Neg.	Neg.	Neg.	

[1] Complement-fixation.

[2] Collodion particle agglutination.

[3] Mm. induration at test-site.

[4] Insignificant degree of reactivity.

[5] Anticomplementary.

Results of tests for humoral antibodies shown in the body of the table were reported by C. C. Campbell. Aliquots of these eight sera failed to fix complement in dilutions of 1:2 and higher when tested in the laboratory of S. B. Salvin, who stated that such serologic results can be interpreted as indicating either no infection, extremely mild infection or an infection of such long duration that humoral antibodies have disappeared.

in the yard and under the house. Chest roentgenograms were taken on all members of the family; both individuals having calcific foci were sensitive to tuberculin as well as to histoplasmin. For purposes of completeness, and even though the entire family seemed to be enjoying excellent health, the victim's sister was admitted to the ward for a thorough diagnostic survey including cultures and smears of bone marrow and peripheral blood; no evidence of infection was found. The positive intradermal test in one dog was intriguing since the test has been said by Prior *et al.* (1949) to be highly specific in these animals. Significant titers of humoral antibodies were not demonstrable, however, and even proof of infection would merely have justified the deduction that dog and infant may have become infected from the same outside source.

In summary, except for intradermal sensitivity to histoplasmin in the mother and her parents, results of contact investigation within the family were negative, nor was there evidence of histoplasmosis in the household pets. The source and mode of infection in the case here reported, therefore, remained unknown.

DISCUSSION

The observation has been made by Hodgson *et al.* (1951) that "authenticated cases [of histoplasmosis] continue to be more numerous in the areas where interest in this condition is greatest." This impression was nicely borne out by the data presented here and by a remarkably similar situation which has since arisen in Cuba: the first two cases of histoplasmosis were recently found there (Sanguily *et al.;* Barquet, in press) just as a study of intradermal sensitivity to histoplasmin was completed (Pardo *et al.*, in press).

The case here reported was noteworthy for several reasons. Although it was not possible accurately to date the beginning of the infant's illness, the age of onset was probably during the first month of life, thus placing it among the youngest thus far recorded (Hodgson *et al.*, 1951). The three previously recognized local infections were in adult men who had immigrated to this region, two from Martinique, French West Indies, one from Canton, China (Darling, 1908). Were they infected before or after arrival here? The case under consideration, occurring in a native-born Panamanian who was never away from the Isthmus, proved that the fungus was endemic here and capable of causing human disease and even death. Finally, confirming as it did the conclusions drawn from the epidemiologic survey, it established the fact that a portion of the local population has developed intradermal sensitivity to histoplasmin as a result of specific antigenic stimulation by *Histoplasma.*

SUMMARY

Results of an investigation designed to ascertain the status of *Histoplasma capsulatum* on the Isthmus of Panama have been presented. The remarkable prevalence of intradermal sensitivity to histoplasmin, the high prevalence of pulmonary calcific foci in histoplasmin-positive, tuberculin-negative individuals, and the striking similarity of these findings to those reported from known histoplasmosis-endemic areas of North and Latin America, seemed to justify the deduction that unrecognized histoplasmosis was endemic in this region.

This deduction was confirmed by the subsequent recognition of a fatal infection in a four month old child from Chilibre, Republic of Panama. The first human infection since the three original cases described here by Darling nearly 45 years ago, it was the first unquestionably indigenous one to be reported from the Isthmus of Panama.

REFERENCES

Barquet Chediak, J., Histoplasmosis in Cuba: report of case, *Arch. Med. Hosp. Universidad Calixto Garcia* (Habana). In press.

Bass, H. E. 1950, Recent advances in knowledge of fungous diseases of the lungs: with special reference to coccidioidomycosis and histoplasmosis, *J. A. M. A.* **143:** 1041.

Beadenkopf, W. G., Loosli, C. G., Lack, H., Rice, F. A., and Slattery, R. V. 1949, Tuberculin, coccidioidin and histoplasmin sensitivity in relation to pulmonary calcifications, *Pub. Health Rep.* **64:** 17.

Campbell, C. C., and Saslaw, S. 1949, Use of yeast-phase antigens in a complement fixation test for histoplasmosis, *Pub. Health Rep.* **64:** 551.

Darling, S. T. 1906, A protozoön general infection producing pseudotubercles in the lungs and focal necrosis in the liver, spleen and lymphnodes, *J. A. M. A.* **46:** 1283.

— 1908, Histoplasmosis: A fatal infectious disease resembling kala-azar found among natives of tropical America, *Arch. Int. Med.* **2:** 107.

Furcolow, M. L. 1949, Development of calcification in pulmonary lesions associated with sensitivity to histoplasmin, *Pub. Health Rep.* **64:** 1363.

Hodgson, C. H., Weed, L. A., and Clagett, O. T. 1951, Pulmonary histoplasmosis: summary of data on reported cases and a report on two patients treated by lobectomy, *J. A. M. A.* **145:** 807.

Mastellari, A. V., Tuberculin and histoplasmin sensitivities and pulmonary calcifications in 468 children in Panama City. Unpublished data.

Pará, M. 1946, Histoplasmosis in Brazil, *Am. J. Trop. Med.* **26:** 273.

Pardo, O. A., Pardo Castello, V., and Tucker, H. A., Histoplasmin, coccidioidin and tuberculin sensitivites in Cuba: a study of 195 patients, *Bol. San. Panam.* In press.

Prior, J. A., Cole, C. R., and Torbet, V. 1949, Evaluation of histoplasmin reactions in detection of naturally occurring histoplasmosis in dogs, *Pub. Health Rep.* **64:** 1562.

Salvin, S. B. 1947, Complement fixation studies in experimental histoplasmosis, *Proc. Soc. Exper. Biol. & Med.* **66:** 342.

Sanguily, J., Fernandez Ferrer, O., and Leon Blanco, F., Histoplasmosis: report of first case from Cuba. *Vida Nueva.* In press.

Tomlinson, W. J., and Grocott, R. G. 1945, Canine histoplasmosis: a pathologic study of the three reported cases and the first case found in the Canal Zone, *Am. J. Clin. Path.* **15:** 501.

Tucker, H. A. 1951, Coccidioidin sensitivity on the Isthmus of Panama: report of tests performed on one thousand patients, *Am. Rev. Tuberc.* **63:** 657.

Tucker, H. A. 1951, Histoplasmin sensitivity in The Panama Canal Zone: A correlated clinicopathologic study of 1,000 patients, with speculations as to the present status of *Histoplasma capsulatum* on the Isthmus of Panama, *A. M. A. Arch. Dermat. & Syph.* **64:** 713.

Tucker, H. A., Histoplasmin sensitivity and pulmonary calcifications among 1,000 residents of the Isthmus of Panama, *Diseases of Chest.* In press.

Personal communications from Drs. E. S. Wedding, T. B. Reynolds and W. J. Bashe, Jr., 1950.

ARSENAMIDE IN THE TREATMENT OF INFECTIONS WITH THE PERIODIC FORM OF THE FILARIA, *WUCHERERIA BANCROFTI**

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We have already reported (Thetford *et al.*, 1948) the apparently successful treatment with arsenamide[1] of six adult negro males infected with the periodic form of *Wuchereria bancrofti* in St. Croix, the Virgin Islands. This paper summarizes similar treatment of twelve additional cases in the same area.

These subjects were, as the previous six, all adult male Negroes who had lived all or most of their lives on St. Croix. They were selected because microfilariae had been found in their night blood. Of the twelve, eight were asymptomatic and four (J. G., J. J., R. D., and E. B.) had right scrotal hydroceles but were otherwise asymptomatic. The microfilariae were counted in thick smears made from 0.1 cc. of venous blood drawn between 10 and 12 o'clock at night. Such counts were made before treatment, immediately after the completion of treatment, and periodically thereafter as the patients were available up to a year after treatment. The final examination included a search for microfilariae, by Knott's method in 1.0 cc. of blood, also taken between 10 and 12 P. M. arsenamide (as its sodium salt in a 2% phosphate-buffered solution, pH 7.0[2]) was administered intravenously in daily doses of 1.0 mgm. (0.2 mgm. arsenic) per kilogram of body weight.

It will be seen (Table 1) that 12 to 15 daily doses of arsenamide resulted in a dramatic reduction in the microfilaria count during the course of treatment. Eight of the twelve persons treated were negative by the end of treatment, and of the remaining, three exhibited a 97 to 99 per cent reduction; only one (J.B.) had as little as 91 per cent reduction. There was no significant change in the microfilaria counts in the next three to four months but at the end of 11 to 12 months no microfilaria were found in the routine smears of any of the patients (0.1 cc. of blood). The examination of 1.0 cc. of blood by Knott's method revealed microfilariae in the blood of only one (F.S.). It may be significant that this was the individual with the highest pre-treatment microfilaria count and one of the three who received only 12 injections of Arsenamide.

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[1] Arsenamide, generic term for Arsphenamide, p-(bis-[carboxymethyl-mercapto]-arsino)-benzamide.

[2] We are indebted to C. K. Banks and A. C. Bratton, Jr., of Parke, Davis and Company, Detroit, for preparing the drug in 4.0 cc. glass sealed ampoules.

Continued post-treatment follow-up would have been worthless since island-wide mass treatment with diethylcarbamazine (Hetrazan) (Hewitt, 1949) began shortly after the 11 to 12 months follow-up and some of our patients reported receiving and taking that drug at that time.

TABLE 1

Number of microfilaria per 0.1 cc. of night blood before and after treatment with 12 to 15 daily intravenous injections of 1.0 mgm. arsenamide (0.2 mgm. Arsenic) per kilogram of body weight

PATIENT AND DATE OF INITIAL INJECTION	NUMBER OF DAILY INJECTIONS	NO. OF MF. PRE-TREATMENT	NO. OF MF. POST-TREATMENT					
			1–4 days	1 mo.	2 mo.	3–4 mo.	9 mo.	11–12 mo.
J. G. 6/6/47	15	33	1	0	0	0		0 0*
E. M. 6/6/47	15	67	0	0	0	0 0		0 0*
S. F. 6/6/47	15	12	0	7	6	2 3		0 0*
J. J. 6/10/47	15	12	0	0	0	0		
F. S. 6/19/47	12	540	9	19		4 1		0 1.4*
C. H. 6/19/47	12	68	0	0 4	3	0 0 0 0		0 0*
J. B. 6/26/47	15	188	17	0 0			0 0*	0 0
R. D. 7/3/47	15	23	0	0		3 1		0 0*
R. A. 7/11/47	15	117	1	0		0 0		0 0*
O. S. 7/14/47	15	28	0	0	0	0		0 0*
C. K. 7/22/47	12	12	0					
E. B. 8/10/47	15	31	0 0			3 1		

* Examination by Knott's method on 1.0 cc. of blood.

Evidence of minor toxicity was seen in seven of the twelve patients. Nausea occurred in six cases with vomiting in four, and was relieved by phenobarbital *per os*.[3] Two patients had loose stools which ceased on the administration of paregoric. Perivascular leakage of the drug on injection produced local inflammatory reaction in two cases which subsided within three days.

Only one of the individuals (C.K.) developed fever; this was evident after the 12th injection and reached 103°F but subsided within 24 hours. Whether this was due to drug toxicity or to protein resulting from the destruction of the worms or some other cause is not clear. One patient (F.S.) developed a noticeable swelling and tenderness of the right side of the scrotum after the fourth injection which subsided by the time treatment was completed. This reaction may have been due to the destruction of the adult worms.

DISCUSSION

The above series of twelve cases now makes a total of 18 adult Negro males infected with *W. bancrofti* on St. Croix, Virgin Islands, who have been treated with arsenamide. For the combined group as a whole, there was approximately a 99 per cent reduction in the total microfilaria count during the course of treatment, and 12 of the 18 became microfilaria negative. The microfilariae continued to fall off during the next year so that only two were positive 11 to 17 months after treatment. Thus it would appear that the adult worms were either killed or sterilized. While it cannot be conclusively proven that they were killed, the evidence all points to that probability. It has been reported (Otto *et al.*, 1950) that three other species of filaria, the adults of which were examined as a result of necropsy or biopsy after treatment, are killed by this drug before there is any evident injury to the microfilariae. The markedly greater resistance of the microfilariae to Arsenamide is shown by the occurrence of living, active, and apparently normal microfilariae within the disintegrating adults of *Dirofilaria immitis*, killed by the treatment of the canine host with this drug (Drudge, 1951). This is in marked contrast to the several reports of the finding of dead microfilariae in living worms following the use of trivalent antimonials. Thus unless the adults and microfilariae of *W. bancrofti* are completely different from those of the other four species in their relative susceptibility to this drug, it seems reasonable to assume that the adults were killed in this group of patients now totaling 18. Furthermore, two of the patients, one each in the present series of twelve and in the previous series of six, developed scrotal swelling and tenderness suggesting probable death of at least some of the adult worms in the lymphatics of that area. Two other patients developed temperatures and another experienced chills which may have been due either to drug toxicity or the release of proteins following the death of the worms.

The drug in therapeutic doses is relatively non-toxic, and the nausea, vomiting, and diarrhea caused by it appear to be readily relieved or prevented by phenobarbital or better by ascorbic acid. The local sclerosing effect of the drug in the perivascular spaces can largely be avoided by care in administration.

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[3] More recent studies in Samoa (Otto, Jachowski, and Wharton, MSS) have shown that ascorbic acid (50 mgm. q.i.d.) relieves or prevents diarrhea, nausea, and vomiting.

REFERENCES

Drudge, J. H.: Arsenamide in the treatment of canine filariasis. *American Journal Vet. Res.* 1952 (In Press)

Hewitt, Redginal: Mass therapy with 'hetrazan' as a control measure for bancroftian filariasis on St. Croix, American Virgin Islands. *Nature* **164** (4183): 1135–1136, 1949.

Otto, G. F. and Maren, T. H.: Studies on the chemotherapy of filariasis. VII. Comparative review of the possible therapeutic agents available for canine and human filariasis. *American Journal Hygiene* **51**: 385–393, 1950.

Thetford, N. D., Otto, G. F., Brown, H. W., and Maren, T. H.: The use of a phenyl arsenoxide in the treatment of *Wuchereria bancrofti* infection. *American Journal Tropical Medicine* **28**: 577–583, 1948.

FILARIASIS RESIDUALS IN VETERANS WITH REPORT OF A CASE OF MICROFILAREMIA[1]

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During World War II, a large number of Marines and Soldiers were exposed to infection with filariasis. About 16,000 were estimated as having contracted the disease (Magath, 1951). This number is highly questionable since the diagnoses were purely clinical in most cases, based on the signs, symptoms and history of exposure. Magath noted that in only a handful of cases were microfilariae demonstrated in the blood and in another small group of cases adult worms were found in sections made of lymph nodes.

In the past year we have examined 87 veterans discharged from service with the diagnosis of filariasis. In none of these did a search of their service medical records disclose a record of microfilariae in the blood or adult worms in the lymph nodes. All had had repeated blood examinations and many had had lymph node biopsies. The medical records in some instances were incomplete and it was not possible to say how many did have objective proof of filariasis prior to discharges, but the number was undoubtedly small since the medical records were complete in most cases.

The great majority of the men served as Marines. Almost all of the group served on one or more of the following islands: American Samoa, British Samoa, Wallis, and Ellice Islands. A very few had served only on Guadalcanal or New Guinea. Symptoms usually developed about 10 to 12 months after the first exposure, which in the majority of cases was on American Samoa. The initial symptoms were fever, swellings, and dull aching of genitals, upper and lower extremities, both unilateral and bilateral. The lymph glands draining the affected parts were always swollen and tender. Usually more than one area was involved. Exercise always aggravated the condition. By history, in nearly all patients objective swelling diappeared within one year after return home.

At the time of our examination, only two veterans showed swelling, one of the right forearm and the other of the left calf. These were minor in extent but objectively demonstrable. The genitals showed no residuals. Potency and fertility were normal. All veterans had palpable non-tender, discrete, "pea" to "navy-bean" size adenitis of femoral, inguinal, and axillary regions. Occasionally the cervical and epitrochlear glands were demonstrable. In our experience, *axillary*

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[1] Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are the result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

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Appreciation is expressed to Drs. J. F. Kessel, T. B. Magath and G. R. Callender for advice in this study; to Ruth Ross for technical assistance and to Dr. J. Greenberg for permission to report the case study.

adenitis was the only significant finding since enlargement of these nodes is quite an unusual finding during routine physical examinations.

The veterans usually complained of dull aching and swelling of the affected regions after exertion. Psychiatrically, all of the men showed evidences of chronic anxiety related to genital function based on "castration fears". These have been the most persistent symptoms elicited.

BRIEF CASE REPORT

F. G., age 26, was examined on September 25, 1950. He had no complaints at the time of examination. He had arrived on American Samoa in the Spring of 1942 with the First Marine Division and stayed several months. Later he was successively stationed on Wallis Island, British Samoa and Guadalcanal. From Guadalcanal he moved to Australia and then home. He had repeated attacks of malaria. He first developed aching and slight swelling of groins, scrotum, arms, and legs, in 1940, in Guadalcanal. The incubation was about 11 months. He was discharged early in 1945, and stated that he still had slight swelling of right leg and right inguinal glands at the time of discharge. This was aggravated by exertion at that time. The veteran stated that all symptoms gradually disappeared after about one year and he has not had any complaints since 1946.

The only physical findings of interest were discrete, non-tender, "pea-size", firm, bilateral axillary and inguinal lymph glands. The blood disclosed an eosinophilia of 11%, the only case in the entire group. Search of his medical records failed to disclose any finding of microfilariae. Biopsy had not been done.

A fresh blood film was taken at 1 P.M. on September 25, 1950, and disclosed two actively motile microfilariae. These retained their motility for about 72 hours at room temperature after which time the slide was accidentally broken. A Wright-stained slide taken at the same time was sent to Tulane University, Department of Tropical Medicine and Public Health, for species identification. The parasites were identified as *Wuchereria bancrofti* by Dr. A. J. Walker, although the sheaths were not definitely seen.

SUMMARY

1. A case of non-symptomatic microfilaremia due to *Wuchereria bancrofti* is reported approximately seven and a half years after exposure to infection.

2. The objective residuals of infection in 87 cases of filariasis in veterans consist of lymph node enlargement in all cases, especially axillary. Rarely, slight swelling of the affected limbs was demonstrable.

3. The subjective symptoms were complaints of dull pain and swelling of the affected parts after exertion, and anxiety related to the genital organs. The patients were not exercised so that it is possible that some of the complaints concerning swelling may have a foundation in fact.

ADDENDUM

Since this paper was submitted for publication, a second case of microfilaremia has been found. This veteran showed no periodicity over a 24 hour study period.

Concentration studies of whole blood with saponin revealed from 4 to 7 microfilariae per cubic centimeter in each of 8 samples, while direct fresh smears and thick drop stains only revealed one parasite in 6 examinations during the same period. The veteran was asymptomatic. Species identification and the concentration studies were done in cooperation with Dominic DeGiusti, Ph.D., Dept. of Zoology, Wayne University.

REFERENCE

MAGATH, T. B. 1951, Present status of filariasis among veterans, *Tropical Medicine News* **8**: 3

ANOPHELES (A.) *EARLEI* VARGAS, 1943, IN MONTANA: IDENTITY AND ADAPTATION TO LABORATORY CONDITIONS (DIPTERA: CULICIDAE)

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Anopheles earlei Vargas and *A. occidentalis* Dyar and Knab, unlike the other dark-winged North American anophelines, possess a coppery spot at the apex of the wing, indicating a close relationship to the members of the Palearctic *A. maculipennis* complex. Vargas (1943, 1944) and Vargas and Matheson (1948) have presented morphological characters in the adult, larva, and pupa by which *A. earlei* may be separated from *A. occidentalis*. These authors suggest that *A. earlei* is found from the New England states to Oregon and Washington while *A. occidentalis* is restricted to the West Coast. "*A. occidentalis*" has been reported from various localities in Canada and Alaska; presumably most if not all of the Canadian records refer to *A. earlei*.

During the past summer the writer was able to spend the month of July at the Rocky Mountain Laboratory in Hamilton, Montana, thus being afforded an opportunity to determine whether the form in this area is *A. occidentalis* or *A. earlei*.[1] Because it is felt that fundamental relationships between closely related species or subspecies are revealed more clearly through genetic and biological studies than through morphological comparisons, the writer was especially anxious to attempt laboratory colonization of *A. earlei*. The purpose of this paper is to present the results of observations on taxonomy, adaptability to laboratory conditions, and certain biological characteristics of *A. earlei* in Montana.

MATERIALS AND METHODS

Several miles north of Hamilton, at a locality known as Tucker's Mill, a good breeding place of *A. earlei* was found, along the margins of a canal leading to a mill pond. At the center, the canal was three to four feet deep; the water moved slowly, except at the margins, where thick floating and emergent vegetation furnished protection to the larvae. The water was clear and very cold, being extremely uncomfortable for wading in tennis shoes. A reading made at 9:30 A.M. on July 11 showed a surface temperature of 16° C. Most of the larvae and pupae utilized in this study originated in this channel, but some additional ones were collected from a low, marshy seepage area about a hundred yards removed from the canal, and in a small semi-permanent pond. The water in the seepage area was cold, and breeding was fairly heavy in certain deeper parts, while that of the shallow pond was considerably warmer. The pond contained plenty of protective vegetation, and the fact that only a few larvae were found in it may have been due to a preference on the part of the ovipositing females for colder waters.

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[1] The author is indebted to Dr. C. L. Larson, Director, and to Dr. C. B. Philip of the Rocky Mountain Laboratory for laboratory space and assistance.

For the taxonomic studies, large larvae and pupae were isolated in separate containers and the adults were bred from them. Thus each adult was associated at least with its pupal skin, and most of them were also associated with the skin of the fourth stage larva.

Adult females were captured underneath bridges near Hamilton; also, several hundred were captured from human bait. Most of the latter were taken near the West Fork Ranger Station, about 15 miles south and west of Darby, Montana. Wild-caught females were isolated in individual test tubes in which a strip of moist filter paper had been placed, for the purpose of obtaining individual egg batches. It should be noted that in nature the females became active at dusk, when the weather was so cool that it was quite uncomfortable to bare the arms and legs for bait.

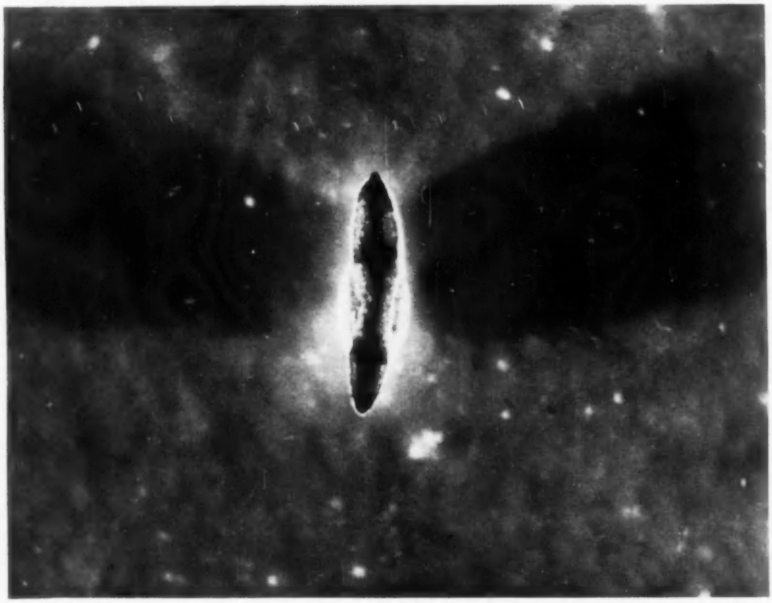

FIG. 1. Egg of *Anopheles earlei* in Montana; dorsal surface, showing transverse dark bands. Photograph by N. J. Kramis.

IDENTITY OF THE MONTANA SPECIES

The identification of the Montana species was based upon a comparison of an adequate series of associated material with the descriptions given by Aitken (1945), Vargas (1943, 1944) and Vargas and Matheson (1948).

The egg. Aitken describes the egg of *A. occidentalis* in California as being light grey in color, and unbanded on the dorsal surface. The egg of the Montana form differs markedly from this in that the dorsal surface is light grey in color, with a dark spot at each pole, and with a dark transverse band near each end of the floats (Fig. 1). The floats contain 17 to 21 float ridges, which are finely corrugated. The ventral surface shows a faint polygonal pattern. Only barred eggs were associated with the Montana species; they were obtained from wild-caught females, from the laboratory colony, and from the breeding water at Tucker's Mill. A single batch of plain grey eggs was obtained from a wild-caught female; adults reared from these eggs proved to be *A. freeborni*.

The larva. Characters used by Vargas and Matheson for separation of larvae of *A. earlei* and *A. occidentalis* are the inner anterior clypeal hairs (i.a.c.), the post clypeal hairs (p.c.), and hair 2 (antepalmate) of abdominal segments IV and V. Comparisons of the number of branches of these hairs as given by the above authors with those exhibited by the Montana specimens are given in Table 1. The 136 inner anterior clypeal hairs of 71 larvae were observed. Although 15 of the hairs were single, in only four of the 71 larvae were both hairs single. The branching of the post clypeal hairs does not appear to be of value as a separation character. Aitken agrees with Vargas and Matheson that the antepalmate hairs of abdominal segments IV and V of *A. occidentalis* are single. If this holds true, the branching of these hairs should be a good key character. Of 278 hairs examined in the Montana specimens, none was single, while 95 per cent of the hairs had three or more branches. Of 74 larvae examined for this character, not one had all four of these hairs 2-branched.

TABLE 1

Comparison of larval hairs of Montana specimens with descriptions of A. occidentalis *and* A. earlei

CHARACTER	BRANCHING ACCORDING TO DESCRIPTIONS		MONTANA SPECIMENS									
	occidentalis	earlei	Total exam.	Distribution according to number of branches								
				1	2	3	4	5	6	7	8	9+
i.a.c.	1	2	136	15	78	31	10	1				1
p.c.	4+	3	136	3	29	38	29	17	6	6	4	4
Hr. 2, IV & V	1	2+	278		14	112	90	40	18	3		1

The pupae of *A. occidentalis* and *A. earlei* differ, according to Vargas and Matheson, in that the lateral spines of abdominal segment VII are sharply pointed and at least seven times longer than broad in the former, while in the latter they are rounded, and at most five times longer than broad. Of 122 Montana pupae examined, 96 had round lateral spines on segment VII, most of which were not over six times longer than broad. In 19 pupae these spines were pointed; in four of the pupae the spines were double, triple, or frayed; and in three they were rounded on one side and pointed on the other. The pointed and branched spines varied in size from five to 20 times longer than broad. Several of the pupae with the longest spines were associated with males possessing *earlei* terminalia.

Without knowing how much variation occurs in the lateral spines of *occidentalis* pupae, it is not possible to be certain as to the validity of this character. Evidently it can not be a certain key separation; however, if the spines on segment VII are used in combination with those on segments V and VI, separation of the two species may be more certain. Many of the specimens which had pointed spines on VII had rounded ones on V and VI; only three had pointed spines on both VI and VII.

The ninth tergite lobes of the male terminalia have been described as being long and narrow in *A. occidentalis* and short and broad with the apex somewhat dilated in *A. earlei*. Of 62 males of the Montana species examined, 33 had ninth tergite lobes which were 1.2 to 2.0 times longer than broad at the base; in 27 these lobes were 2.1 to 2.5 times longer than broad; one was 2.7 and one was 3.3 times longer than broad. The latter was associated with larval and pupal skins typical of *A. earlei*. The lobes of most of these specimens were expanded apically (Fig. 2).

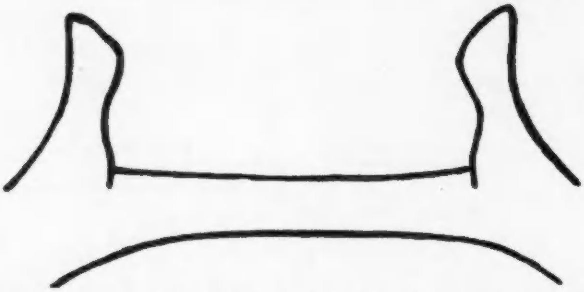

FIG. 2. Ninth tergite lobes of male terminalia of *Anopheles earlei* in Montana.

Vargas and Matheson also describe differences in the size and scaling of the wings of *A. occidentalis* and *A. earlei*. Without material of *A. occidentalis* for comparison, the present writer did not feel that he was able to interpret these characters in the Montana specimens, and so the wings were excluded from the present study. All the wings, of course, possessed the coppery fringe spot at the apex.

LABORATORY REARING OF *A. EARLEI*

All of the specimens used for establishing the colony in the Rocky Mountain Laboratory were collected as larvae and pupae at Tucker's Mill. A few of the eggs found here were also placed in the breeding pans. Several hundred adults emerging from this material were examined under the microscope, and all exhibited the coppery spot at the apex of the wing; thus it became apparent that there was no contamination by *Anopheles freeborni*.

The larvae were placed in small white enameled trays. About 200 larvae per tray seemed to be the optimum concentration, but a shortage of containers made it necessary to double or even triple this number in some of the trays containing younger stages. Overcrowding did not seem to cause undue mortality, but it probably did reduce the size of the adults. The inside of each tray was lined with a sheet of paper toweling; ridges in the toweling extending up to the water surface formed resting places for the larvae, and it may be that the toweling absorbs deleterious waste products. Bates (1947) utilized mud in the rearing of his colony of *A. darlingi*, and the writer has used toweling in the larval pans of his other *Anopheles* colonies for many years, as this procedure seemed to result in the production of larger and healthier pupae.

The larvae were fed small amounts of Fleischmann's yeast, pressed firmly on a strip of filter paper which was floated on the water surface by means of an underlying strip of wax paper. They were also fed finely ground laboratory animal food. Three types of the latter were available: mouse pellets, guinea pig pellets, and dog food, all manufactured by mills in Montana. Since there was no time to determine which of these might be superior, the larval food was made by pulverizing equal parts of the above materials. This food was dusted over the surface of the water six or more times daily.

Pupae were harvested daily, transferred to a dish of fresh water, and placed inside the colony cages.

Three types of cages were used for the adults. The smallest, with two opposite sides in the form of a trapezoid, had an inside capacity of 647 cubic inches; the second measured one by one by one and a half feet, and the third, one and a half by one and a half by two and a half feet. Mating took place readily in each of these three cages. It was noted that about a minute after one's arm was inserted in a cage for the purpose of feeding the females, the males became active and began to swarm in the center of the cage. This occurred even in the smallest cage when only three or four males were present, and at any time of the day. Males became especially active at dusk, and at this time those in the largest cage swarmed regardless of the presence of a vertebrate host. Pairing seemed always to take place during flight; after union the female would come to rest on the side of the cage, with the male suspended head down by means of the clasping organs; contact was maintained for 30 to 60 seconds. Dissection of a few females showed that this process resulted in fertilization.

The females were fed upon the arms of human hosts either by day or night. The first blood meal was taken readily, but the females appeared to be very reluctant to feed again.The seeming reluctance of the females to take more than one meal of blood may have been the reason for the long delay in the appearance of colony eggs. The first blood feeding took place in the colony cages on July 7, but eggs did not appear until July 25. Meanwhile adults were added to the cages daily, and engorgement continued to take place. The numbers of females that took blood varied from four or five to 40 per day. The second batch of eggs appeared on July 30. Both of the above egg batches were fertile.

At the end of July all of the adults were transferred to the largest of the three cages. The total population numbered about a thousand individuals, including a hundred or more females with abdomens distended with eggs. No more adults were added to the cages.

From August 1 to September 3, Dr. C. B. Philip observed 19 additional ovipositions. Each batch contained about 100 to 350 eggs, and the total number deposited was estimated to be approximately 4000. Most of these eggs were sent to Baltimore, and the fact that only several hundred of them produced living larvae is believed to have been due to the hazards of the trip rather than to infertility.

About 200 adults were obtained from the eggs sent to Baltimore. These adults mated and fed, but produced only two small batches of eggs. A second generation of laboratory-bred adults were obtained from these eggs, but they were too few in number to perpetuate the colony.

DISCUSSION

The separation of *A. earlei* and *A. occidentalis* should be placed upon a more solid basis through a study of an adequate series of the latter species. Although the morphological characters of the Montana specimens were quite stable, it is not impossible that considerable variation may occur in *A. occidentalis* in the antepalmate hairs of the larva, the lateral spines of the pupa, and in the shape of the ninth tergite lobes of the male. One might also note that all of the writer's specimens were taken in one small area and they may represent a small interbreeding population with less variation than may be exhibited by *A. earlei* in other parts of its range. Nevertheless, because of the agreement of these characters with the descriptions of *A. earlei*, and especialy because of the differences in the eggs, it is concluded that the Montana species is *A. earlei* and not *A. occidentalis*.

The barred egg of *A. earlei* indicates a very close relationship between this species and *A. maculipennis* (type species) of Europe. That these two are not the same is indicated by the fact that *A. earlei* mates readily in a cage less than a half a cubic foot in size, while *A. maculipennis* requires a very large cage in which to swarm (Bates and Hackett, 1939). Eggs of *A. earlei* were obtained in only the two larger cages; however, successful swarming and mating took place in the smallest cage. Because of the long delay in the appearance of the first colony eggs, the adults were removed from the smallest cage in order to built up a higher concentration of them in the larger cages. It is possible that had these adults been allowed to remain in the small cage, they, too, would have produced fertile eggs.

It should not be too difficult to colonize *A. earlei* successfully. The larvae are easily reared. Although the total number of eggs obtained was small as compared with that produced by colonies of other *Anopheles* species, laboratory rearing of successive generations might result in adaptation to cage conditions with an increase in egg production. It is felt that the failure of the writer's colony was due to the attempt to transfer it too quickly from Montana to Baltimore. Not only was the sub-colony too small, but the males did not become active at dusk. The marked activities of the caged males in Hamilton may be an adaptation to the long twilight of the area inhabited by the northern species.

SUMMARY AND CONCLUSIONS

The identity of the Anopheles species with the pale-tipped wing in the vicinity of Hamilton, Montana, is considered to be *A. earlei*. The egg of this species differs from that of *A. occidentalis* in that it possesses two dark transverse bars on the dorsal surface. *A. earlei* thus appears to be closely related to *A. maculipennis* (type species) of Europe, but differences in cage behavior of the male is evidence that these two are not the same. *A. earlei* appears to be adaptable to colonization; the writer's colony failed perhaps because of the inability to send enough living eggs to Baltimore from Montana.

REFERENCES

AITKEN, T. G. 1945, Studies on the anopheline complex of western America, *Univ. Calif. Publ. in Ent.* **7**: 273–364.

BATES, M. 1947, Laboratory colonization of *Anopheles darlingi*, *Jour. Nat. Mal. Soc.* **6**: 155–158.

BATES, M., AND HACKETT, L. W. 1939, The distinguishing characteristics of the populations of *Anopheles maculipennis* found in southern Europe, *Verh. VII. Int. Kongr. Entom.*, Berlin, 1938, **3**: 1555–1569.

VARGAS, L. 1943, *Anopheles earlei* Vargas, 1942, n. sp. norteamericana del grupo maculipennis, *Bol. Of. Sanit. Panamer.* **22**: 8–12.

VARGAS, L. 1943a, El "Grupo *maculipennis*" del. Nuevo Mundo y el *Anopheles earlei*, *Rev. Inst. Salub. Enferm. Trop.* **4**: 279–284.

VARGAS, L. 1944, Algunas consideraciones sobre *Anopheles occidentalis* Dyar y Knab, 1906, *Rev. Inst. Salub. Enferm. Trop.* **5**: 215–220.

VARGAS, L., AND MATHESON, R. 1948, Estado actual del *Anopheles earlei* Vargas (1943) y *Anopheles occidentalis* Dyar & Knab (1906) con claves para larvas, pupas y adultos del llamado complejo *maculipennis* de Norteamerica, *Rev. Inst. Salub. Enferm. Trop.* **9**: 27–31.

NOTES ON *ANOPHELES EARLEI* AND OTHER AMERICAN SPECIES OF THE *ANOPHELES MACULIPENNIS* COMPLEX

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In the period 1936–1941 many fundamental contributions were made to the classification of the anopheline mosquitoes in this country. Bradley (1936), King (1939), and their associates worked out the *Anopheles crucians* complex in the Southeast and published a number of papers dealing with the larvae and adults, (King, Bradley, and McNeel, 1939; King and Bradley, 1941). In the West, Aitken (1939) published his studies on the *Anopheles maculipennis* complex, describing *Anopheles freeborni*, the important malarial mosquito of the West, and definitely separating it from *occidentalis* and *aztecus*. He also recognized two subspecies in the *pseudopunctipennis* complex: *pseudopunctipennis pseudopunctipennis* and *pseudopunctipennis franciscanus* (Simmons and Aitken, 1942). As a result of all this work, during the war years most medical entomologists recognized thirteen species of *Anopheles* in the United States, with three distinct species in the *maculipennis* complex in North America following the work of Aitken (1939): *occidentalis* in Canada, northern United States, and the Pacific Coast; *freeborni* in western United States and Canada; *aztecus* in Mexico.

Vargas (1943) described *Anopheles earlei* basing it on characters of the male which appeared subject to so much variation that most entomologists were inclined to consider it a synonym of *occidentalis* (Aitken, 1945). However, subsequent studies by Vargas (1943a), Matheson (1948), Vargas and Matheson (1948) and Penn (1949) indicate that the adults, larvae, and especially the pupae of *earlei* are quite distinct from those of *occidentalis*. There are therefore fourteen species of *Anopheles* mosquitoes in the United States, and four species of *Anopheles* in the *maculipennis* complex in North America, as will be discussed in this paper.

In the "*maculipennis* complex" in North America, the adults may be separated easily into two groups based on the type of wings: *freeborni* and *aztecus* have all of the wing scales uniformly brownish, whereas *occidentalis* and *earlei* have the fringe at the tip of the wing of a definite silvery, coppery, or golden-yellow color and four dark spots in the wing as in *A. quadrimaculatus*.

In the larvae (Plate 1, figs. 1–4), *aztecus* is most distinct, having huge, widely separated inner clypeal hairs. The other three species have these hairs smaller and more closely spaced. *Earlei* typically has the inner clypeal hairs forked beyond the middle, with two to six branches beyond the middle in the majority of specimens. The heads of *freeborni* and *occidentalis* are quite similar with single, closely spaced inner clypeal hairs. However, the antepalmate hairs on the fourth abdominal segments are very different in these species (Plate 2, figs. 5–8). The antepalmate hair is typically single in *occidentalis*, double in *freeborni*, and multiple in *earlei* and *aztecus*. There is, therefore, a considerable difference between the larvae of *earlei* and *occidentalis*, the adults of which are quite similar in general coloration.

In the pupae, the studies of Vargas and Matheson (1948) and Penn (1949) have shown striking differences (Plate 3, figs. 9–12). *Occidentalis* is the most distinct with a very long, slender spine in the posterior-lateral corner of the seventh abdominal segment. In *freeborni* and *aztecus*, this hair is shorter, curved, and pointed, while in *earlei* it is short, stout, straight, and blunt. Thus, it will be seen that *occidentalis* and *earlei* which are quite similar in the adult stage are very distinct in pupal characters.

With regard to distribution, the four species are also very distinct (Plate 4). *Aztecus*, as its name implies, is Mexican. *Freeborni* occurs in western United States west of the Rockies and in an area east of the Rockies in western Texas, the eastern part of New Mexico, and most recently in southeastern Colorado. There are specimens in the Communicable Disease Center Entomological Museum collected by L. D. Beadle at Fort Lyon, Caddoa and Boone, Colorado. *Occidentalis* is found along the Pacific Coast region, perhaps east as far as the Rockies. *Earlei* appears to occur in northern United States and southern Canada east of the Rockies, according to Matheson (1948). The writer has examined specimens from Maine, New York, Michigan, Minnesota (south to Winona, Minnesota), Wheatland, Wyoming, and Glacier National Park, Montana (F. C. Harmston). It is reported from Hamilton, Montana by L. E. Rozeboom. Much additional study is needed to delimit more accurately the geographical distribution of these four species, particularly where their ranges overlap.

Biologically there appear to be considerable differences between the four species. *Freeborni* is considered a good feeder on human beings and a vector of malaria in parts of New Mexico, California, Utah, and Oregon, according to Aitken (1945). *Aztecus* is considered a vector of malaria in parts of Mexico (Downs and Bordas, 1949). It is practically the only anopheline found in certain malarious areas. *Occidentalis* seldom feeds on human beings, and therefore is considered as a poor vector of malaria in California. *Earlei* is a vicious biter as Matheson and Belkin (1943) have pointed out. The writer can attest from personal experience in northern Minnesota that *earlei* will bite man in the bright sunlight. It will also invade houses and feed on man at all hours during the night, thus rivalling *Mansonia perturbans* in blood-thirstiness. It is a northern species, however, and probably has little or no importance as a vector of malaria.

SUMMARY

In North America it now appears that the *maculipennis* complex is composed of at least four species; *occidentalis* Dyar and Knab, *aztecus* Hoffman, *freeborni* Aitken, and *earlei* Vargas, which can be recognized in the majority of cases on adult, larval, and pupal characters.

ACKNOWLEDGMENTS

The writer is indebted to many U. S. Public Health Service entomologists for advice and specimens during the course of this study, particularly G. H. Bradley,

F. E. Lyman, M. H. Goodwin, F. C. Harmston, and L. D. Beadle. R. M. Bohart of Davis, Calif., supplied specimens of *Anopheles occidentalis* from California; H. T. Peters reared a series of all stages of *Anopheles earlei* from Bemidji, Minnesota; and W. G. Downs, Mexico City, Mexico, the material of *Anopheles aztecus*.

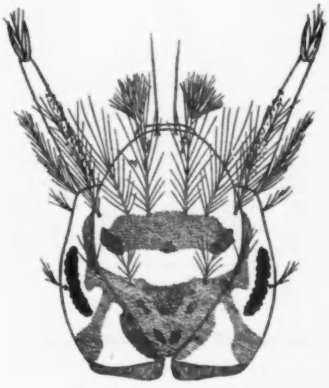
REFERENCES

1. Aitken, T. H. G. 1939, The *Anopheles maculipennis* complex of western America. *Pan. Pac. Ent.* **15:** 191–192.
2. Aitken, T. H. G. 1945, Studies on the anopheline complex of Western America. *Univ. Cal. Pub. Ent.* **7** (11): 273–364, 39 figs.
3. Bradley, G. H. 1936, On the identification of the mosquito larvae of the genus *Anopheles* occurring in the United States (Diptera, Culicidae). *South. Med. Journ.* **29:** 859–861.
4. Downs, W. G. and Bordas, E. 1949, Hallazgo de *Anopheles aztecus* Hoffman naturalmente infectado con plasmodium, en la zone de Xochimilco, D. F. *Rev. Inst. Salubridad y Enferm. Trop.* **10** (4): 312–325.
5. King, W. V. 1939, Varieties of *Anopheles crucians* Wied. *Amer. Jour. Trop. Med.* **19:** 461–471.
6. King, W. V., Bradley, G. H. and McNeel, T. E. 1939, The mosquitoes of the southeastern states. *U. S. D. A. Misc. Publ. No. 336*, pp. 1–90.
7. King, W. V. and Bradley, G. H. 1941, General morphology of *Anopheles* and classification of the Nearctic species of *Anopheles*, pp. 63–70. Distribution of the Nearctic species of *Anopheles*, pp. 79–87. A symposium on human malaria. *Pub. No. 15 Amer. Assoc. Adv.Sci.*, Wash., D. C.
8. Matheson, R. 1948, The status of *Anopheles earlei* Vargas (1943). *Proc. 35th Meet. N. J. Mosq. Ext. Assoc.* 69.
9. Matheson, R. and Belkin, J. 1943, Notes on *Anopheles occidentalis* in central New York. Proc. 30th Ann. Meet. N. J. Mosq. Ext. Assoc. 7–11.
10. Penn, G. H. 1949, Pupae of the Nearctic anopheline mosquitoes north of Mexico. *Jour. Natl. Mal. Soc.* **8** (1): 50–69, 14 figs.
11. Simmons, J. S. and Aitken, T. H. G. 1942, Anopheline mosquitoes of the northern half of the western hemisphere and of the Philippine Islands. *Army Med. Bulletin No.* **59:** 1–213.
12. Vargas, Luis. 1943a, El "Grupo *Maculipennis*" del Nuevo Mundo y el *Anopheles earlei*. *Rev. Inst. Salub. Enf. Trop. Mex.* **4:** 279–286.
13. Vargas, Luis. 1943, *Anopheles earlei* Vargas, 1942, n. sp. norteamericana del grupo maculipennis. *Bol. Oficina Sanit. Panamericana*, **22** (1): 8–12.
14. Vargas, Luis and Robert Matheson. 1948, Estado actual del *Anopheles earlei* Vargas (1943) y *Anopheles occidentalis* Dyar and Knab (1906) con claves para larvas, pupas, y adultos del llamodo complejo *maculipennis* de Norteamerica. *Rev. Inst. Sal. Enferm.* Trop. Mex. **9** (1): 27–33, 2 pl.

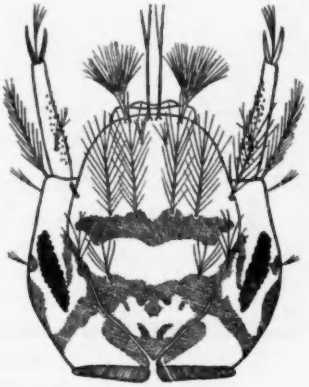
EXPLANATION OF PLATES

Plate I. Dorsal view of heads of fourth stage larvae of

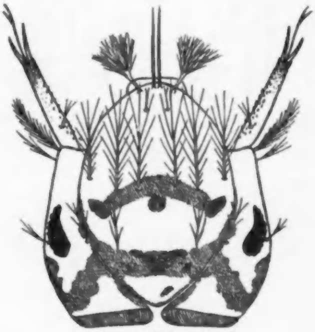
1. *Anopheles aztecus*
2. *Anopheles earlei*
3. *Anopheles freeborni*
4. *Anopheles occidentalis*



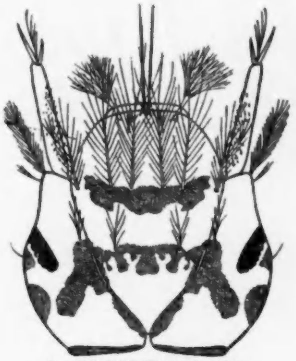
1. AZTECUS



2. EARLEI



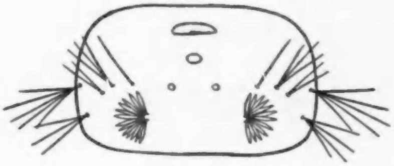
3. FREEBORNI



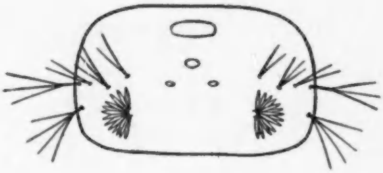
4. OCCIDENTALIS

PLATE II. Dorsal view of fourth abdominal segment of fourth stage larvae of

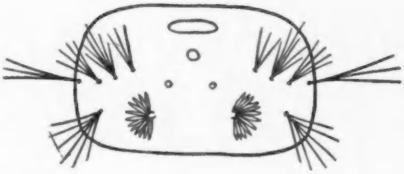
5. *Anopheles occidentalis*
6. *Anopheles freeborni*
7. *Anopheles earlei*
8. *Anopheles aztecus*



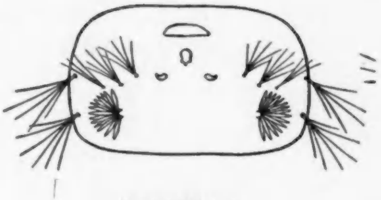
5. OCCIDENTALIS



6. FREEBORNI



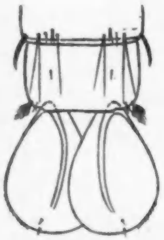
7. EARLEI



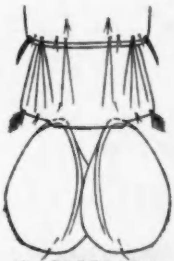
8. AZTECUS

PLATE III. Dorsal view of terminal abdominal segments of pupae of

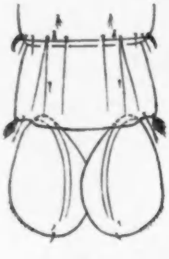
9. *Anopheles occidentalis*
10. *Anopheles freeborni*
11. *Anopheles aztecus*
12. *Anopheles earlei*



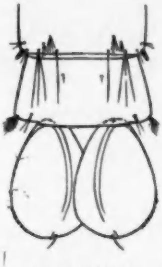
9. OCCIDENTALIS



10. FREEBORNI

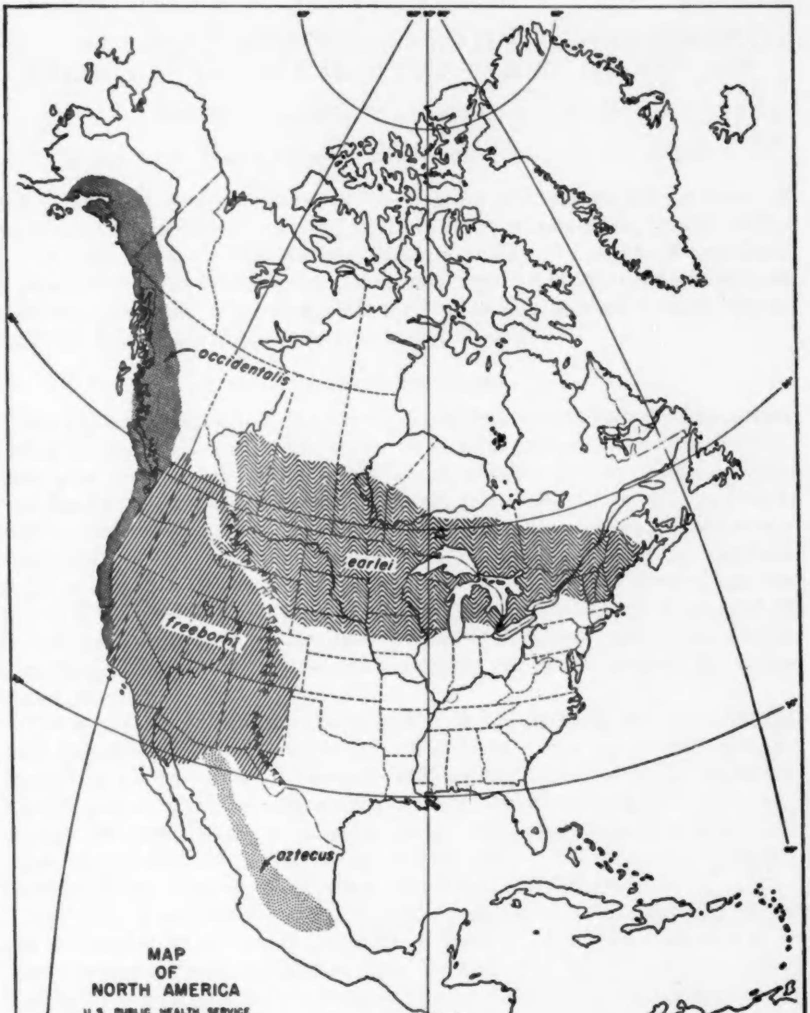


11. AZTECUS



12. EARLEI

Plate IV. Distribution map of *Anopheles maculipennis* complex in North America


occidentalis
earlei
freeborni
aztecus
MAP
OF
NORTH AMERICA
U. S. PUBLIC HEALTH SERVICE
MALARIA CONTROL IN WAR AREAS
ATLANTA, GEORGIA
SCALE OF MILES
0
200
400
600 MI.

PROGRESS REPORT OF EPIDEMIOLOGIC APPRAISAL OF REPORTED MALARIA IN THE UNITED STATES DURING 1949 AND 1950

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A description of the initiation of the program of epidemiologic appraisal of malaria in the United States in 1947 has been presented elsewhere (Quinby 1950). At the 1950 meeting of the National Malaria Society, the results of appraisals of reported malaria for 1949 and preliminary data for the first nine months of 1950 were presented. Since then, data for 1950 cases appraised through March 1951 have been assembled and analyzed for this paper.

SCOPE OF COVERAGE

The primary function of the appraisal program was to measure the actual incidence of malaria. Later this became an integral part of the Malaria Surveillance and Prevention Program of the Communicable Disease Center and the state health departments concerned. Although this program is carried out principally through the investigation of officially reported cases, more and more emphasis has been given to finding and confirming unreported malaria. The final stages of the malaria eradication program should be directed toward the discovery of the residual foci. Moreover, it is anticipated that the determination of malaria endemicity may depend upon the results of such investigations. Consequently, the adequacy of malaria appraisal is the key to the success of the eradication program.

The epidemiologic personnel participating in the program have not changed from that reported in 1949 except through addition of new areas to the progam. Texas began to participate in October 1949, and the Tennessee Valley Authority Malaria Control Division collaborated actively from May through August 1950, adding Tennessee to the area under study. Thus, appraisal of reported and rumored malaria has been under way in 1950 in 7 States: Alabama, Arkansas, Georgia, Mississippi, South Carolina, Tennessee, and Texas.

The ratio of appraised cases to reported cases for the 13 eradication States has increased from .08 for 1948, and .09 for 1949, to .32 for 1950 (appraisals and cases are not mutually inclusive). Since Texas is the only State in the nation reporting malaria by numbers of cases without identification of the patient, a comparison based on the other 12 eradication States is more appropriate. With Texas excluded, the ration of appraisals to reports rose from .13 in 1948, and .33 in 1949, to .73 in 1950.

More detailed investigation of individual cases has been made possible by the numerical decrease in reports and the additional experience of the appraisers. Efforts have been made not only to confirm reports, but to establish the differential diagnosis of any illness with which it may have been confused. In most states this appraisal program has been broadened to include appraisals of typhus and other febrile illnesses.

REPORTED MALARIA IN 1949 AND 1950

Table 1 demonstrates the continued decrease of reports in the 13 eradication States and the rest of the United States. Texas contributed more than two-thirds of the reports for the nation during 1950. A comparison of numbers of cases reported to the state health departments with those forwarded to the National Office of Vital Statistics shows that some malaria reports submitted to the states were not considered by them to be sufficiently valid for inclusion in the official reports.

TABLE 1

Appraisals of malaria occurring in 1949 and 1950*

STATE	REPORTS†		APPRAISALS			
	1949	1950	1949		1950	
			Reports (to State)	Rumors	Reports (to State)	Rumors
Alabama	134	87	26	3	5	17
Arkansas	323	52	115	3	61	4
Florida	43	7				2
Georgia	91	37	90		29	1
Kentucky	21	6				
Louisiana	21	5	1		1	
Mississippi	72	64	94	3	65	1
Missouri	4	2				
North Carolina	53	37	2			1
Oklahoma	92	93				
South Carolina	242	86	151	2	67	
Tennessee	35	18	2		1	9
Texas	2,881	1,605	12	10	288	9
13 States Total	4,012	2,099	493	21	517	44
Other States and D. C.	219	128	1	0	0	3

* Through March 1951, by State, Tennessee Valley Authority and Public Health Service epidemiologists.

† From National Office of Vital Statistics.

Increasing efforts have been made to detect unreported cases. Thousands of rumored cases have been followed up by a large force of malaria eradication personnel and other interested local health service personnel. Laboratory reports have been investigated. From these sources only 65 appraisals materialized. Nine of them were "positive"; that is, confirmed by laboratories approved by the state health department. Of these 9, one case was introduced into Arkansas from Panama. Another was a patient with her sixth asymptomatic parasitemia found in monthly blood surveys during the last 6 years at the Santee-Cooper Reservoir in South Carolina. Of the other 7, from Texas, 1 was a South Pacific relapse, 1 was an indigenous relapse, and 5 were declared primaries. Four of these primaries are based on incomplete military records and the other has a history of a visit to Mexico.

THE OCCURRENCE OF MALARIA IN 1949 AND 1950

Table 2 shows the distribution of 60 positives (Quinby 1950), discovered through March 1951, that were reported or were known to have occurred in 1949. Of these, 17 have been accepted by the State epidemiologists as primaries of origin within the United States, but only 3 of them without reservation. Fourteen were accepted with reservations as follows:

TABLE 2

Appraisals of malaria occurring during 1949*

STATE	TOTAL APPRAISALS	CLASSIFICATION OF APPRAISALS								
		Positive in Laboratory Approved by State					All Other (1)			
		Total	Indigenous		Introduced	Other†	Presumptive	Doubtful	Not Malaria	Incomplete
			Primary	Relapse						
Ala.	29	3		1	2		18	8		
Ark.	118	3	1		1	1	12	13	3	87
Fla.	0									
Ga.	90	19		2	14	3	13	58		
Ky.	0									
La.	1	1	1							
Miss.	97	4	3		1		34	58	1	
Mo.	0									
N. C.	2	2	1	1						
Okla.	0									
S. C.	153	13	7	3	1	2	89	51		
Tenn.	2						1	1		
Texas	22	15	4	1	10		1	6		
Total	514	60	17	8	29	6	168	195	4	87

* Through March 1941, by State, Tennessee Valley Authority, and Public Health Service epidemiologists.

† Arkansas, by transfusion; Georgia, source of infection unknown; South Carolina, 1 induced, and 1 by transfusion.

5 had some slight doubt as to validity of laboratory confirmation;
3 patients had been in malarious areas of the Orient less than 2 years previous to onset;
4 were based on incomplete military data;
1 was complicated by a history of 5 transfusions; and
1 was a patient with her sixth asymptomatic infection detected since 1944.

This leaves 3 unquestioned primaries for 1949.

The distribution of the 26 positives which were appraised or were known to have occurred in 1950 is shown in Table 3. Six of these are considered primaries by state epidemiologists, but only 2 without reservation. Two are based on confirmations of questionable validity. One was discovered as a complication of tularemia and may have been a relapse. Another was said by friends to have been in Mexico recently, though he denied it. This leaves 2 unquestioned primaries for 1950.

No two primaries for either year were associated in time, place, or person.

The evaluation of malaria control has long been based on the incidence of primary malaria, particularly in children under one year of age. During 1949 and 1950, the youngest child with confirmed malaria was 6 years of age.

TABLE 3

Appraisals of malaria occurring during 1950*

STATE	TOTAL APPRAISALS	CLASSIFICATION OF APPRAISALS								
		Positive in Laboratory Approved by State					All Other (1)			
		Total	Indigenous		Introduced	Other	Presumptive	Doubtful	Not Malaria	Incomplete
			Primary	Relapse						
Ala.	22	1			1		8	13		
Ark.	65	1			1		7	22	30	5
Fla.	2							2		
Ga.	30						3	27		
Ky.	0									
La.	1	1	1							
Miss.	66	3		1	1	1†	11	45	3	4
Mo.	0									
N. C.	1							1		
Okla.	0									
S. C.	67	4	4				11	29	1	22
Tenn.	10						2	8		
Texas	297	16	1	5	9	1‡	9	240	11	21
Total	561	26	6	6	12	2	51	387	45	52

* Through March 1951, by state, Tennessee Valley Authority, and Public Health Service epidemiologists.

† By transfusion

‡ Induced

CONCLUSIONS

(1) Within the areas covered by the appraisal program during 1949 and 1950, 86 positives were found; 23 of these are regarded as primaries, only 5 of which are unquestioned.

- (a) For 1949, 60 positives were discovered; of these, 17 are regarded as primaries, only 3 of which are unquestioned.
- (b) For 1950, 26 positives were found; of these, 6 are regarded as primaries, only 2 of which are unquestioned.

(2) The coverage of the appraisal program in time and area is not sufficiently extensive to constitute a complete measurement of incidence in the nation or in the 13 eradication states.

(3) The lack of association between cases within the eradication states studied indicates very little malaria transmission.

Acknowledgements: The authors are indebted to many state, Tennessee Valley Authority, and Public Health Service epidemiologists for their participation in the program, as well as to many local health service personnel and the large force of malaria eradication personnel who constituted a case-finding screen.

REFERENCE

Quinby, Griffith E. 1950, Epidemiological appraisal of reported malaria in the United States from 1947 through 1949, *Jour. Nat. Mal. Soc.* **9**: 397–400.

TRENDS IN MALARIA CONTROL PROGRAM OF THE TENNESSEE VALLEY AUTHORITY[1]

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In the early 1930's, as soon as TVA began planning for development of the Tennessee River, it was confronted by a malaria problem. Despite advances up to that time in knowledge concerning control of malaria, the disease still constituted one of the most important public health problems of the southeastern United States, and the relationship of impoundages and malaria was well documented. Reservoir impoundments in the region had proved in virtually every instance to be ideal breeding grounds for anopheline mosquitoes, and some had resulted in serious epidemics of malaria. The TVA project envisioned creation of a system of artificial lakes potentially totalling over 600,000 acres of water area and over 10,000 miles of shoreline. Impoundage of this magnitude in an area where malaria existed to a serious degree presented an unprecedented control problem. Moreover, the problem was intensified by the multipurpose character of the reservoir system which was to be operated for flood control, navigation, and the generation of power and would require flexibility in water level management to meet the needs of all three purposes. The development of the malaria control program and operating experience for the period 1934–1947 has been reported upon previously (Bishop and Hinman, 1948). It is the purpose of this report to summarize significant developments in the program since 1947.

From the beginning, the TVA malaria control program has emphasized measures directed at control of the vector, *A. quadrimaculatus*. That is, the primary effort has been to prevent malaria transmission by controlling the density of the vector. Principal elements of the program are reservoir preparation, water level management, permanent shoreline improvement, shoreline and drainage maintenance, and larviciding. Limited use has been made of house mosquito proofing and DDT premises spraying; and in a section of one reservoir (Kentucky), special malaria control easements were purchased, prohibiting nighttime occupancy in the one-mile zone during the anopheline breeding season.

RESERVOIR PREPARATION

There have been no significant changes in TVA reservoir preparation practices since the last main stream reservoir, Kentucky, was impounded in the fall of 1944. All reservoirs constructed since that date have been headwater storage projects located in mountainous terrain which required only basic clearing and a minimum of marginal drainage. On two of the most recently impounded projects, the final marginal conditioning operation was omitted because of the light re-growth following initial clearing and the generally steep and exposed nature of the shorelines.

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[1] Prepared for presentation at meeting of National Malaria Society, Chicago, Illinois, November 14–17, 1951.

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WATER LEVEL MANAGEMENT

Water level regulation is still the most effective single mosquito control measure, and control needs have been fitted successfully into reservoir operating schedules required for flood control, navigation, and power. The schedule on the main river reservoirs includes: (1) raising the water above normal maximum summer level in late winter or early spring and then returning it to that elevation in order to strand the winter's accumulation of drift and flotage; (2) maintaining the water at the normal maximum summer elevation during the spring growth period to retard marginal vegetation; (3) cyclical fluctuation of the water levels about 1 foot at weekly intervals during the late spring and early summer in order to kill mosquito larvae by stranding them on shore or exposing them to natural enemies such as top minnows; and (4) combining a seasonal recession of about 0.1 foot to 0.2 foot per week with cyclical fluctuation in order to minimize the area and duration of contact between vegetation and water. This has been found to insure effective control of mosquito larvae during the peak of the mosquito breeding season in the summer and early fall. On the storage reservoirs effective mosquito control is usually obtained through the use of wide seasonal recession of water level. Of the twenty-four TVA owned reservoirs in the system, water level management alone provides satisfactory mosquito control on sixteen reservoirs; on two reservoirs only occasional and limited larvicidal operations are required; and regular seasonal larvicidal operations and other measures are scheduled on only six reservoirs.

The trend of present thinking in TVA with regard to water level schedules for mosquito control is to extend the constant pool phase during late spring and early summer in the interest of vegetation control even though extensive larviciding may be necessary for a short time to prevent heavy mosquito production. Thus, when it becomes necessary to initiate cyclical fluctuation or seasonal recession, lowering the reservoir level 0.5 foot to 1.0 foot withdraws the water from the zone of heaviest growth invasion. These are considered as refinements within the basic water level management schedules, and application depends upon mosquito control appraisals made currently each season.

PERMANENT SHORELINE IMPROVEMENT

The program of shoreline alterations to eliminate the most serious mosquito breeding areas in the lower main stream reservoirs, which was initiated in 1947, is continuing. This program was initiated after successful experience in Kentucky Reservoir where extensive shoreline improvements were completed prior to impoundage. These shoreline improvements consist mainly of diking and dewatering and deepening and filling. The latter method consists of deepening the lower half of shallow mosquito breeding areas and placing the excavated earth on the upper portion of the area. Diking and dewatering consist of building dikes to separate large shallow areas from the lakes and installing pumps to remove water which accumulates behind the dikes. Approximately 9100 acres, 50 per cent of the total 18,200 acres proposed for permanent shoreline improvement measures in TVA reservoirs, have been completed. Of the 9100 acres completed, 4960 acres are included in ten diking-and-dewatering projects: eight in Kentucky Reservoir and two in Wheeler Reservoir. No additional diking-and-dewatering projects are planned. Approximately 4200 acres (32 per cent of total) of deepening and filling have been completed, and 9100 acres remain to be done. Completion of the permanent shoreline improvement program was originally scheduled for fiscal year 1953, but work has been curtailed to accommodate to more acute construction needs.

Except for brief periods of unfavorable water levels, excellent mosquito control has been achieved in the deepened and filled areas without supplementary larviciding. Except in one reservoir, Hales Bar, where construction requirements at the dam prevented usual water level schedules for several years following completion of the deepening and filling work, mosquito control operations in the deepened and filled areas have been negligible, consisting only of minor drainage maintenance. In contrast, experience with the diking-and-dewatering projects, particularly in Kentucky Reservoir where the areas inside the dikes were not cleared, has been generally unfavorable, both from the standpoint of mosquito control and of operating and maintenance costs. In some of the larger projects, it has not been possible to keep all parts of the area dewatered completely enough to prevent heavy production of mosquitoes; and supplementary larviciding has been required in such situations. Seepage has been a major problem in one project, and parts of the project have been blocked off, cleared, and, by means of sluice gates, water level fluctuation for mosquito control provided. Two diking-and-dewatering projects in Wheeler Reservoir were completed after impoundage. Because the areas were cleared as a part of the preimpoundage preparation of the reservoir, there is more latitude in planning pumping schedules than is the case with the uncleared areas in Kentucky Reservoir. The Wheeler projects are operated as lateral impoundages, with the pumps being used to provide mosquito control water level fluctuations independent of the main reservoir.

The dewatering projects in Kentucky Reservoir are currently under study to determine steps that might be taken to improve their effectiveness for mosquito control and to decrease pumping and maintenance costs.

SHORELINE AND DRAINAGE MAINTENANCE

Cumulative beneficial effects of water level management and of annual marginal growth removal operations have been effective in limiting the zone of marginal vegetation invasion to 1½ to 2 feet below normal pool elevation. The area of annual removal of marginal vegetation has been reduced from a peak of approximately 33,400 acres in the fall of 1942, even before Kentucky Reservoir was impounded, to approximately 6,300 acres in the fall of 1950. After careful analysis of operating experience, field observations of shoreline conditions, and review with the state health departments concerned, decision was made this year to discontinue cutting of vegetation in the upper six inches of the normal fluctuation zone. This decision was based on the fact that little benefit could be derived from the cutting operation in this zone due to resprouting, which occurs in the early spring before the reservoirs are completely filled, and the relatively short period of time that the water is in the cut over zone under usual water level schedules. This change in shoreline maintenance practice eliminated the necessity for cutting of marginal vegetation in Pickwick and Wilson Reservoirs, and sharply reduced the acreage in other reservoirs. In Kentucky Reservoir, where the predominant growth along the margins is buttonball, *Cephalanthus occidentalis*, which is well established in the zone about 1½ feet below normal pool elevation, decision was made not to carry out any growth removal operations this fall. This decision was reached after field studies had shown that successive annual cutting of buttonball in the reservoir could not be expected under prevailing water level schedules to push the encroachment of the plant farther landward. Thus, this fall only 3,150 acres of marginal cutting are scheduled on all TVA reservoirs, 50 per cent of the acreage in 1950 and 9.5 per cent of the maximum annual acreage which was cut in 1942.

These reductions in growth removal operations will result in increased larvicidal requirements next spring, but this will be more than offset by savings in shoreline maintenance expense.

Herbicidal operations are a relatively small part of the control program, yet extremely important. Formulations of 2,4-D have been found to be effective in the control of objectionable reservoir plants, including black willow, trumpet vine, lotus, parrot's feather, water primrose, water willow weed, cocklebur, and giant ragweed. They are used routinely for lotus control in reservoir areas and for control of willows, smartweed, lotus, and parrot's feather in marginal drainage ditches. Effective herbicidal treatment has not yet been developed for alligator grass, buttonball, and cattail. Of these, alligator grass and buttonball are of major concern; and studies to develop practical herbicidal treatments for these plants are to be continued. In the case of buttonball, because of the acreage involved the herbicide, to be practical, must be effective in small enough quantities to make application by aircraft (helicopter) feasible.

Maintenance of existing marginal drainage systems is necessary to assure maximum effectiveness of water level fluctuation for mosquito control. Improved methods for using dynamite on ditch maintenance have been developed and have largely supplanted conventional hand and machine methods.

LARVICIDING

Although principal reliance for mosquito control has always been upon biological or naturalistic control measures, supplementary chemical control measures have been and continue to be required. The principal chemical control measure, larviciding, has undergone progressive change since its first use by TVA in 1933. During the ten-year period, 1933–1943, developments in larviciding techniques consisted principally of improvements in equipment for hand and boat application of kerosene and black oil larvicidal mixtures and airplane application of Paris green dust. During World War II, as DDT and other new and more effective insecticides became available, TVA immediately tested the more promising ones to determine their effectiveness in the control of anopheline mosquitoes and to develop satisfactory formulations and equipment for their application. Airplane application of DDT for mosquito control was first carried out by TVA in cooperation with the U. S. Department of Agriculture in 1943. Later, as the result of a cooperative project undertaken by TVA, the U. S. Department of Agriculture, and research units of universities working under grants from the Office of Scientific Research and Development, a new type of DDT solvent was used and an exhaust generator was developed for the production of an aerosol or mist-type spray which could be readily adapted to small airplanes. Because of the small quantity of DDT thermal aerosol (0.1 lb. DDT per acre) necessary for satisfactory control and its more effective penetration of vegetation, this development increased tremendously the effectiveness and economy of airplane distribution. Subsequently, spray equipment was devised which reduced the rate of application necessary for effective mosquito control to .05 lb. of DDT per acre. DDT applied by airplane as a spray has completely replaced all other methods of larviciding. Recent investigations have demonstrated that helicopters can be used satisfactorily for application of larvicides under conditions prevailing on TVA lakes; and since TVA already uses helicopters for transmission line patrol, joint use for both transmission line patrol and malaria control is being explored. Such an arrangement would permit more effective and economical utilization of equipment and pilots than is presently the case.

During the early years of TVA impoundages, there were certain limited areas in which adequate mosquito control was impracticable with any methods then available. It was therefore decided to employ mosquito proofing of houses within these areas as a temporary measure until more permanent measures could be applied. Following the wartime demonstration of the effectiveness of wall sprays of DDT in eliminating flies and mosquitoes, spraying methods were developed which field tests demonstrated to be practicable in the TVA area. Thereafter, with the approval of appropriate state health departments, DDT residual spraying of all dwellings and other structures of premises was substituted for mosquito-proofing maintenance. Beginning with the 1951 season, routine use of premises spraying in these areas was discontinued, and the areas included in the regular anti-larval program. This change was made possible by the cumulative beneficial effects of water level management in controlling marginal vegetation, progress on permanent shoreline improvement, and increased effectiveness of larviciding techniques. Residual spraying equipment and materials are being maintained in standby condition so that premises spraying may be utilized if emergency situations should indicate the need for it.

The same factors cited above, which led to discontinuation of premises spraying as a routine control measure, have been effective in reducing larvicidal requirements. In fiscal year 1938, Wheeler Reservoir alone (1,063 miles of shoreline) required approximately 95,000 acres of airplane dusting and 4,800 miles of boat and hand larviciding. In fiscal year 1951, only 5,600 acres of airplane larviciding were required in this reservoir; and for the entire TVA system of lakes with over 10,000 miles of shoreline, only 38,100 acres of larviciding were required. Maximum utilization of water level management for marginal growth control within the limitations of primary operating requirements of TVA main stream reservoirs is dependent upon the availability of efficient larvicidal measures that can be used to control mosquito production and permit extension of the constant pool phase well into the mosquito breeding and plant growing season. For this reason and because efficient larvicidal measures need to be continually available for use in the event of temporary disruption of naturalistic control measures which might result in emergency situations, the apparent acquired resistance to DDT of *A. quadrimaculatus* observed recently is a matter of serious concern. A comprehensive investigation of this phenomenon was undertaken last year and is continuing. From analyses of data from airplane larvicidal experiments and tests during the years 1945–1951 inclusive, it appears that there has been a change in the susceptibility of *A. quadrimaculatus* larvae to DDT; and in 1951, the application rate of DDT under average conditions of vegetative cover had to be increased from .05 lb. per acre, used successfully in previous years, to 0.1 lb. per acre, in order to secure larvicidal effects in the field comparable with prior experience. There is some evidence that this apparent development of resistance to DDT may be related to increased use of chlorinated hydrocarbon insecticides on cotton dusting operations in the vicinity of TVA reservoirs. A detailed report on these and related studies is being prepared by the staff.

PROGRAM APPRAISAL

In order to appraise the effectiveness of the TVA control program, TVA and the state health departments cooperate in making routine inspections of designated mosquito collecting stations during the mosquito season, and carry out investigations of malaria morbidity in areas adjacent to the reservoirs. Average annual station counts (number of female *A. quadrimaculatus* per inspection) for the period 1945–1951, are summarized as follows:

RESERVOIR	1945	1946	1947	1948	1949	1950	1951
Kentucky	50	11	6.9	6.7	7.5	3.9	15
Pickwick	17	2.2	1.7	2.7	2.5	0.9	2.7
Wilson	4.9	0.6	1.0	1.4	1.1	0.1	0.8
Wheeler	35	15	6.8	18	4.0	0.6	9.5
Guntersville	20	5.3	1.9	4.1	3.5	1.6	5.3
Hales Bar	15	2.2	0.2	3.1	2.5	2.5	3.1
Chickamauga	3.2	0.4	0.2	2.4	0.6	0.4	4.3
Watts Bar	1.0	1.0	0.9	1.4	1.3	1.2	1.8
Fort Loudon	0.1	0.1	0.2	0.0	0.0	0.0	0.2
All Reservoirs	22	5.9	3.4	5.6	3.5	1.7	7.4

Blood film surveys have been carried out each year during the period 1934–1950 in areas around TVA reservoirs where malaria transmission potentials were believed to be greatest. The trend of malaria incidence as measured by these surveys has closely paralleled the downward trend of malaria parasitemia in the southern United States. In 1934, approximately 28 per cent of the 6,720 slides examined were positive for malaria parasites; whereas less than 1 per cent were positive on all surveys after 1941. All slides from surveys in 1949 and 1950 were negative. Seasonal blood film surveys were discontinued beginning with the 1951 season when decision was made to rely upon field epidemiological investigations to determine whether malaria transmission was occurring in the vicinity of TVA reservoirs. These investigations were carried out in collaboration with state and county health departments and coordinated with the general malaria surveillance program of the Communicable Disease Center, U. S. Public Health Service. No cases of confirmed indigenous malaria were discovered during the 1951 season. Four confirmed cases of malaria proved, upon investigation, to be of Korean origin.

PRESENT OUTLOOK

Prospects appear favorable for continuation of present or lower levels of malaria endemicity in the southern United States despite the recurring hazard of foci of infection afforded by cases of foreign origin, provided preventive and control measures are not reduced or abandoned unwisely. So long as significant anophelism can occur in the Tennessee Valley and other regions of the southern United States, there will be a hazard of malaria transmission. Obviously, the hazard will diminish in proportion to the number of individuals in the population haboring malaria parasites, but the hazard will remain. There have been outbreaks of malaria in the northern states of this country and in other locations after many years of freedom from the disease. Those interested in malaria control in the United States should keep in mind, among other considerations, at least two factors which exert an influence upon the epidemiology of the disease.

The first is the effect of the economic status and density of rural populations on malaria prevalence. The past fifteen years have witnessed relatively great prosperity of rural people in the southern states. This has made possible the widespread use of antimalaria drugs and of insecticides, and has been associated with general improvement in rural housing. Without doubt, these developments have contributed in large measure to the decline of malaria in the south. At the same time, particularly during 1942–1945, there has been migration of rural people to urban communities, and evidence of a reversal of this trend is not apparent. Should this period of prosperity be followed by a depression, previous experience suggests that there would be migration from urban to rural communities; and it is likely that there would be less inclination by people exposed to mosquito bites to spend money for antimalaria drugs. Such a set of circumstances might well contribute to a recrudescense of malaria.

The second factor is the evidence that the effectiveness of DDT as an insecticide has diminished over the past few years. Data already referred to above suggest that *A. quadrimaculatus* may be developing tolerance to DDT. That this phenomenon has been observed in other insect species is widely known. While it is too soon to appraise the problem with regard to anophelines, it must be kept in mind.

A third factor is of importance insofar as malaria prevention in the vicinity of TVA lakes is concerned. This is the extensive and rapidly expanding use of the lakes for recreational purposes. There are thousands of private summer cottages along the reservoir shorelines and numerous public facilities with overnight accommodations. Their use by vacationers and indigenous populations in close association is one more contact potential among those which could weld together the chain of malaria transmission.

In view of these considerations, no change in the basic malaria prevention program of TVA is contemplated within the foreseeable future. Principal reliance will continue to be upon measures directed to the control of anopheline production on TVA lakes together with epidemiologic surveillance for early detection of any case of malaria which might occur, and application of such emergency measures as might be indicated. Through frequent reappraisal of operating programs and reservoir conditions, both the scope and intensity of anopheline control measures will be modified in keeping with experience and the results of investigations.

A great deal has been learned during the past fifteen years concerning the control of anopheline production on impounded waters. Not the least important fact is that naturalistic controls and, indeed, much of the foundation for artificial controls should be a part of the initial planning of the impoundment. The current national interest in development of water resources suggests that stream impoundments will continue to be planned, some of them undoubtedly in potentially malarious areas. Malaria in the United States is now at an unprecedented low. This fact should be no cause for neglecting the need for building control potentials into new water management projects. An outbreak of man-made malaria would be a high price to pay for the dubious distinction of not taking into account the basic engineering, biological, and epidemiological advances so far attained in the control of one of man's most ancient and debilitating enemies—malaria.

REFERENCE

Bishop, E. L. and Hinman, E. Harold 1948, Malaria control trends on impounded waters of the Tennessee Valley Authority, *Jour. Nat. Mal. Soc.* 7: 12–22.

THE SCORPION PROBLEM IN RIBEIRÃO PRETO, SÃO PAULO, BRAZIL. NOTES ON EPIDEMIOLOGY AND PROPHYLAXIS

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Ribeirão Preto is one of the most important cities in the interior of the State of São Paulo, Brazil, and is situated about 200 miles northeast of the capital at an altitude of 1,600 feet. It has 15,500 buildings, and a population of approximately 77,500. During the past several years the number of scorpions scattered throughout the city had begun to attract attention, but the accidents caused by the stinging of the arachnid were not recorded until the installation of the Municipal Emergency Service.

In July, 1950, the Serviço de Profilaxia da Malária (Malaria Service) of the State of São Paulo was asked to study and to endeavor to solve the problem. The accidents were evidently on the increase, and the population was becoming more and more concerned, especially when two deaths occurred within a short space of time.

EPIDEMIOLOGY

An epidemiological investigation was begun in June, 1950, in order to secure accurate information with regard to the local scorpions. The results are shown in Table 2.

Only four species were found: *Tityus serrulatus*, *Tityus bahiensis*, *Bothriurus bonariensis*, and *Bothriurus magalhaensis*. *Tityus serrulatus* greatly predominated and was eminently urban and domestic in habits. The others were generally found frequenting more rural zones, and usually outside of houses. It was the common belief of the local population that the scorpions were hiding and breeding in the inlets of the rainwater drainage system, but careful investigation did not confirm this opinion. Possibly under certain conditions these pipes might provide a temporary refuge, but during the hot, dry season they would not appear to be suitable retreats for scorpions which prefer darker and more humid hiding places. Arsenical and sulfur gases were released in sections of the drains where people said they had often seen scorpions without producing any signs of them.

Some 10,000 scorpions have been captured since then, mainly inside houses, in bathrooms, bedrooms, kitchens and also in rubbish heaps in the yards. In the latter case, they were found very often in piles of bricks. A good place to look for scorpions was in the narrow space under the houses, from which they had access to the house through cracks in the floors. Possibly the wood carried into the homes for fuel may have furnished transport for scorpions. Contractors engaged in tearing down houses stated that they rarely found scorpions in attics. Undoubtedly when temperatures reach a certain degree, scorpions tend to migrate to cooler areas. The contractors, like everyone else, often spoke of finding scorpions by the thousands, but in our investigations, we never found more than 16 in any one place at a given time, and then only on a few occasions.

Table 1 gives the data supplied by the Emergency Service on accidents through scorpion stings in the population. The number of persons reported stung was 1,331 over a 7 year period, and increased yearly until the beginning of the campaign, while the number of fatal cases, though small, has doubled in the last five years. While the trend evidently gives cause for alarm, the absolute numbers are not in excess of those reported from some other areas. Stahnke reports an average of about 3,000 scorpion accidents a year, with 60 or more deaths over a period of twenty years, in the State of Arizona, which has a population of 750,000.

TABLE 1

Data on persons recorded as stung by scorpions in Ribeirão Preto, January 1945–December 1951

(Supplied by local Emergency Service)

YEAR	PERSONS STUNG*			REGION OF BODY AFFECTED						REC'D ANTI-SERUM	DEATHS		
	Oct.-Mar. (Summer)	Apr.-Sept. (Winter)	Total	Hand	Foot	Arm	Leg	Head and Trunk	Not specified		No.	Age	Month
1945	27	21	48	28	13	4	3	—	—	4	1	3 yr.	Oct.
1946	37	28	65	33	16	5	3	1	7	4	1	9 mo.	Nov.
1947	56	27	83	40	14	4	1	4	20	8	1	3 mo.	Oct.
1948	109	87	196	86	32	8	11	9	50	4	0	—	—
1949	188	142	330	203	56	20	14	13	24	35	2	5 yr.	Sept.
												7 yr.	Oct.
												2 yr.	Jul.
1950	205	208	413	242	89	34	22	22	4	9	2	6 yr.	May
1951	117	79	196	105	40	20	16	10	5	—	1	—	Aug.
Total	739	592	1,331	737	260	95	70	59	110	64	8	—	—
Per cent	56	44	100	55	20	7	5	4.5	8.5	6.5	0.6	—	—

* See graph on page 511.

TABLE 2

Number of scorpions captured, by year, and species

Species of scorpion

PERIOD	TITYUS SERRULATUS	TITYUS BAHIENSIS	BOTHRIURUS BONARIENSIS	BOTHRIURUS MAGALHAENSIS	UNCLASSIFIED	TOTAL
1949	2,053	177	6	—	20	2,256
1950	4,536	258	155	—	1,026	5,975
1951	1,405	344	15	14	76	1,854
Total	7,994	779	176	14	1,122	10,085

A tabulation of the victims by age, sex and color did not reveal any very significant differences; 635 males and 630 females were stung, and the age group 16–46 apparently suffered the most, being possibly the most active and exposed, but no conclusions can be drawn since the age-composition of the population is not known. What seems clear is that the younger the individual, the greater the risk of a fatal outcome. Eight deaths occurred, all in young children from 3 months to 7 years old (the age of one is unknown) (Table 1).

As for the region of the body in which the sting was received, Table 1 shows that the hands were principally involved, and that hands and feet together accounted for 75% of all the cases. No part of the body however was exempt. For various reasons only a few (6.5%) received specific antiserum but a discussion of this measure does not fall within the scope of this paper. It was a question which had its controversial aspects and will be discussed at a later date.

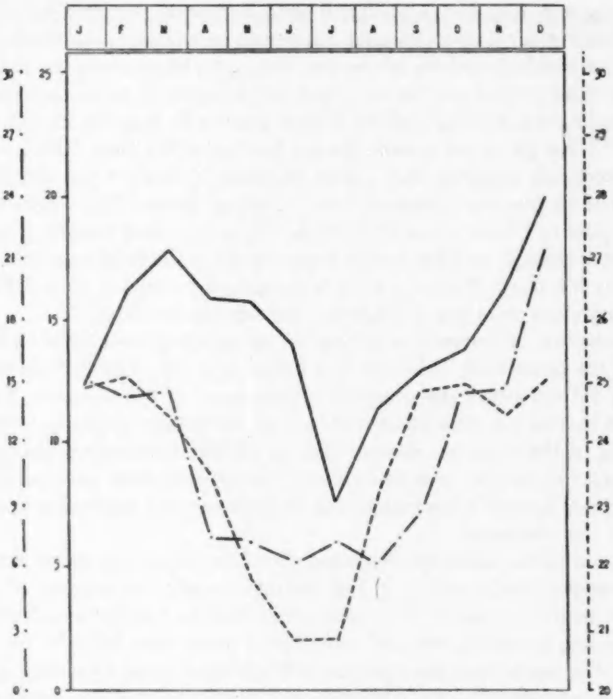
The time of day when the stings were received is of interest, as shown by the following records:

Time of day	*No. of persons stung*
6 a.m. to noon	424
Noon to 6 p.m.	444
6 p.m. to 11 p.m.	355
11 p.m. to 6 a.m.	103
Time unknown	5
	1,331

The scorpion has nocturnal habits and was evidently quite active until 11 p.m., but the preponderance of stings occurred in daylight hours, being almost equally distributed between morning and afternoon. This must have been due to disturbance of dormant scorpions by human activities. The reverse situation on a diminished scale occurred after 11 p.m. when the scorpions attacked human beings at rest, particularly the children who go to bed early.

The season of the year was a very important factor in scorpion accidents. As shown by the accompanying graph there is a clear correlation between temperature, rainfall and number of persons stung by scorpions. The majority of the deaths (seven out of eight) occurred from August to November which is the beginning of the rainy season when the temperature is warming up to summer. There is some support for the belief that there is a seasonal variation in the toxicity of the venom. Craig and Faust (1951) say that "the venom is most potent during the warm breeding season." Magalhães (1946) noted that the virulence of bites is greater in August, September and October (spring in Brazil) than in April, May and June. In both laboratory and clinic there was an appreciable difference in the pure venom of *T. serrulatus* and *T. bahiensis* between the cold and the hot months. That this might however also be a quantitative difference is suggested by the fact that it took a gland and a half, or even two, in July to provide the same amount as a single gland from the same species in October. In addition to a possible seasonal variation in the toxicity of the venom, it is reasonable to suppose that the number of accidents increases as scorpion activity is renewed after the torpidity induced by winter. Season would have little effect to increase the scorpion population since it takes scorpions five years or longer to complete the life cycle.

Ribeirão Preto, São Paulo Sate, Brazil
1945–July 1950



Average number of scorpion stings correlated with temperature and rainfall, by month and year.

——— Average number of stings
- - - - - Average temperature (Centigrade)
— · — Average rainfall (mm)

PROPHYLAXIS

Two insecticides were used in an experimental attack on the scorpion: a 10% ortho-BHC powder (Gammexane) and a 30% mother solution of DDT (dichlorodiphenyl-trichoroethane).

BHC in a dosage of 0.25 grams per square meter of surface gave very satisfactory results. The scorpions were driven from their hiding places and those that were captured for observation died within 24–48 hours, following a period of intoxication which became evident within an hour of exposure. Later, the dosage was reduced to 0.13 grams per square meter which proved equally fatal, although the period of intoxication and death were delayed.

DDT was used in a 4% solution, applied in a dosage of 2 grams per square meter of surface. This treatment also drove the scorpions from their hiding places but was certainly less immediately toxic than the gamma isomer of BHC. Signs of poisoning were manifest only after 6 hours and death occurred within 48 to 72 hours. However our present data do not permit us to draw conclusions as to the comparative residual toxicity of the two toxicants. Since scorpions may go into hiding for three or four months at a time, any insecticide, to accomplish eradication, should have a residual toxicity of from four to six months. In anti-mosquito work, DDT has produced a more lasting residual effect than BHC.

The insecticide spraying had a dual purpose, to destroy the scorpion in its habitat and to prevent scorpions from entering houses. Thus favorite hiding places in piles of bricks, wood or rubbish, the space under houses or the cracks in walls were treated, and the results were evident in the dead scorpions observed in the next few days. Within a week after spraying a school with 3,000 pupils, 29 dead scorpions were found inside and outside the building.

The protection of houses was attempted by spraying insecticide on boundary walls and the outer walls of houses to a height of 5 feet. The short period of the campaign did not prove the complete effectiveness of the measures, but it was significant that in the areas treated there were no further accidents. A year later a checking of the situation showed that in all the areas where the insecticide was sprayed the number of persons stung was reduced when compared with the previous year. Longer observation will be necessary to determine the residual efficacy of the toxicants.

In addition to the measures described above, an effort was made through the local newspaper, radio and by public lectures to stir the interest of the local population in the campaign. Forty collecting stations for scorpions were located in pharmacies, to which over 200 scorpions a week were brought by the local residents. The teachers of the grammar schools were urged by means of lectures to enlist the cooperation of the pupils and a prize was publicly awarded by the mayor to the student collecting the largest number of scorpions. Displays illustrating and explaining the campaign were held in various sectors of the city and were attended by the population in large numbers.

SUMMARY

A survey of the city of Ribeirão Preto in the State of São Paulo, Brazil, revealed four species of scorpion: *Tityus serrulatus*, *Tityus bahiensis*, *Bothriurus bonariensis*, and *Bothriurus magalhaensis*. The first species was predominant, and because of its urban and domestic habits, was responsible for the majority of accidents to human beings through scorpion stings, of which 1,331 were reported over a period of 7 years, with 8 deaths in small children.

Ortho BHC, and DDT in solution were used in an experimental eradication campaign and both were effective. BHC was more rapidly lethal, but the period of observation was too short to permit an evaluation of the comparative duration of residual toxicity of the two poisons.

REFERENCES

CRAIG, C. F., AND FAUST, F. C. 1951, Clinical Parasitology, 5th Ed., Lea and Febiger, Philadelphia, page 641.

MAGALHÃES, OCTAVIO DE, AND GUIMARÃES, R. 1941, Algumas observações sôbre acidentes pela picada dos escorpiões (*T. serrulatus*), *Brasil Med.* 55: 461–465.

III. HISTOPATHOLOGICAL STUDIES ON CUTANEOUS REACTIONS TO THE BITES OF VARIOUS ARTHROPODS[1]

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In the course of investigations (Goldman, *et al.*, Rockwell *et al.*, in press) on the mechanisms of the bites of various arthropods in the skin of man, histopathologic study of the area of the bite was found to be an important part of the work. The clinical reaction to such a bite can be characterized usually as a localized area exhibiting redness, swelling, heat, itching and pain in varying degrees of intensity. Since these are the classical clinical concomitants of tissue response to injury i.e. inflammation, it is perhaps not too surprising that the histology of this inflammatory reaction has been taken for granted and that few specific studies relating to early insect bite reactions are available in the literature (Brown *et al.*, 1938; Gordon *et al.*, 1948). The emphasis in medical literature has been on the descriptive pictures of late reactions associated especially with chronic nodular lesions (Ash *et al.*, 1945; Allen, 1948; Winer *et al.*, 1941; Trager, 1939). Moreover, there are few detailed studies correlating the histopathology of the cutaneous lesions with the degree of sensitivity of the individual to the bite of a particular arthropod (Brown *et al.*, 1938).

PROCEDURE AND TECHNICS

In this and previous studies an attempt has been made to correlate the histopathologic changes with clinical manifestations of the bite and some investigative data, such as the allergic background of the individual, the location of the bite, the duration of the feeding of the arthropod and miscellaneous factors such as the variations and response due to the use of various types of local or systemic medication. Biopsies of bite reactions in man only will be presented in this report.

The subjects of this investigation consisted of hospitalized patients and members of the attending and resident staff of the hospital who voluntarily exposed themselves to one or more of the following arthropods: *Aedes aegypti, Anopheles quadrimaculatus, Xenopsylla cheopis, Cimex lectularius, Pediculus humanus, Amblyomma americanum* and *Eutrombicula alfreddugesii*. Some of these were obtained, as in our previous studies, from W. V. King and later W. C. McDuffie, Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, Orlando, Florida. *Anopheles quadrimaculatus* were obtained from Dr. Leon Schmidt, Malaria Research, Christ Hospital, Cincinnati. Our most detailed studies at the present time are related to the bites of mosquitoes. Exposure to

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[1] This investigation was supported by a Research Grant from the Division of Research Grants and Fellowships of the National Institutes of Health, United States Public Health Service.

the arthropods were made at predetermined intervals, the bite areas were marked with India ink, and the bites were subsequently biopsied for histologic study. A detailed clinical description of the bite reaction was done at the time of the biopsy. In several instances, serial biopsies were taken at regular intervals after a simultaneous series of bites on a single individual. The biopsies which were usually from the arm or back, were taken with the cutaneous punch or by scalpel excision after infiltration with procaine. Deep biopsies were necessary in order to study the details of the bite reaction in the subcutaneous tissue. In many instances an attempt was made to include in the skin section the entire arthropod or a portion of the biting apparatus. The proboscis of the feeding mosquito was clipped with small curved scissors. Infiltration of the skin with procaine prior to the bite killed the *Pediculus humanus* in situ. In the case of the tick, collodion was dropped on the feeding arthropod. It was not possible with our current technics (including cyanide crystals) to kill the flea in the act of feeding.

Biopsied tissue was generally placed on paper immediately after removal to avoid curling during fixation. Tissues were routinely fixed in 10 per cent formalin and imbedded in paraffin after dehydration with serial alcohols and chloroform. The blocks were then sectioned serially and stained with hematoxylin-eosin and Goldnere's modification of Masson's trichrome stain. For the studies of the insects *in situ*, in the skin, the biopsied tissue including the insect or its parts was placed on paper and fixed for a period of two to six hours in the Carnoy or Carnoy-Lebrun fixing solution. The tissue was then placed in 95 per cent alcohol and in several cases was treated for a period of twenty-four hours with Bouin's fluid to minimize tissue hardening. Subsequently the tissues were carried through graded alcohol dehydration with passage through chloroform and paraffin. Serial sections were then made. The value of the serial section technics in biopsies of arthropod bites is emphasized so that the analysis of the reaction will be certain to include the immediate area of the bite where the tissue changes are most severe. If examination of the biopsy is confined to the periphery of the reaction, the histological picture will be incomplete.

HISTOPATHOLOGIC PATTERNS OF REACTIONS TO BITES

As a general rule most clinical reactions to the bites of arthropods are characterized by an immediate wheal. Subsequently a papular or tuberculin type of inflammatory response develops after a varying interval of time. The essential histologic characteristics of the immediate reaction are the irregular "puncture canal" in the epidermis and a marked edema of the superficial dermis which is present not only in the immediate area of the bite but also at a considerable distance. Edema may or may not be present in the epidermis adjacent to the actual area of the bite puncture. In the dermis, in addition to the edema there is a marked vaso-dilatation and a perivascular leukocyte infiltration which is usually of a polymorphonuclear type and is most prominent in the center of the bite area.

With the time increase of the inflammatory reaction, there is an increase in the intraepidermal changes with edema, a decrease in the intensity and extent of the edema in the dermis and a distinct increase in the quantity of the perivascular cellular exudate. There is a gradual change in the quality of the cellular response with the neutrophiles decreasing, lymphocytes and eosinophiles becoming more prominent. In some cases in which the clinical appearance was that of a small papule representing the late stage of the insect bite reaction, the cellular response histologically extended far beyond the limits of the clinical papule and deeper than anticipated from clinical examination alone.

The intensity of the reaction, in all likelihood, varies with the sensitivity of the individual. Biopsies of *Aedes aegypti* bites in two individuals with moderate reactions were done at definite intervals following the bites. Thirty minute biopsies showed edema of the upper corium and the beginning of a perivascular infiltrate of polymorphonuclear leukocytes, eosinophiles and lymphocytes. In six hours there was an increase in the intensity and in the spread of the inflammatory reaction with increased edema and increased perivascular infiltrate. Biopsies after twenty-four hours showed a decrease in the edema with no additional vascular response but an increased infiltrate composed of lymphocytes and eosinophiles. At forty-eight hours there was persistence of mild edema but a diminishing infiltrate with an increased percentage of lymphocytes and histiocytes. At five days edema had subsided and the infiltrate consisted merely of a few lymphocytes. Biopsies of a one day old macular reaction to *Aedes aegypti* bites showed a slight perivascular infiltrate composed mainly of lymphocytes and occasional eosinophiles and polymorphonuclear leukocytes. A three day old papulo-vesicular mosquito bite reaction showed marked subepidermal edema

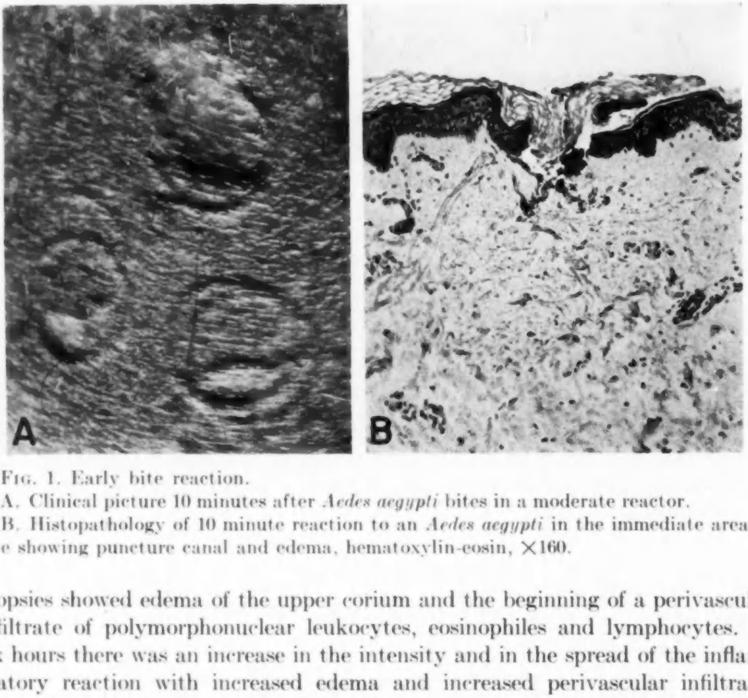

FIG. 1. Early bite reaction.

A. Clinical picture 10 minutes after *Aedes aegypti* bites in a moderate reactor.

B. Histopathology of 10 minute reaction to an *Aedes aegypti* in the immediate area of bite showing puncture canal and edema, hematoxylin-eosin, ×160.

with vesicle formation and a dense perivascular infiltrate composed predominately of lymphocytes. A three day old reaction to *Anopheles quadrimaculatus* in a sensitive patient with clinical reactions up to 15 cms. in diameter showed a dense inflammatory infiltrate extending deeply into the subcutaneous fat composed of numerous eosinophiles, polymorphonuclear leukocytes, lymphocytes, histiocytes, and an occasional plasma cell. In this case, as well as in another severe reactor, the eosinophile response was most striking. Our observations suggest that the severe clinical reactions are accompanied by an inflammation in which epidermal and dermal edema is more marked and the infiltrate is more dense, more extensive, and contains a greater proportion of eosinophiles and polymorphonuclear leukocytes than does the milder clinical reactions to bites.

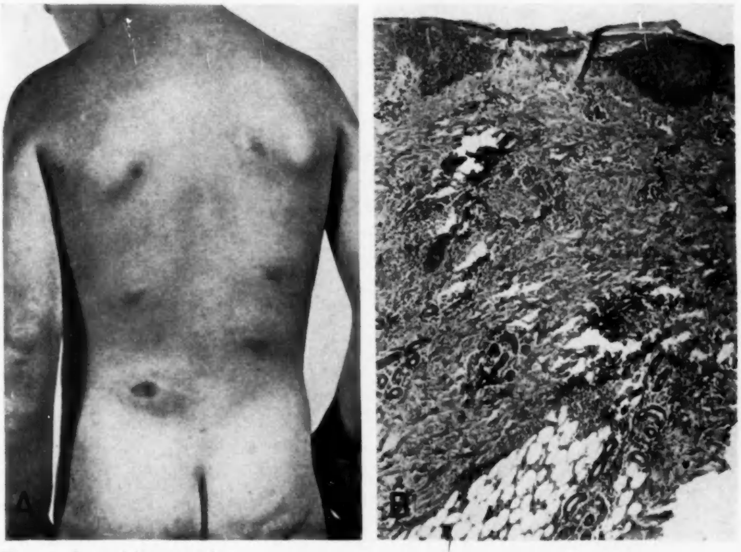

FIG. 2. Late bite reaction.

A. Clinical picture 2 days after 6 *Anopheles quadrimaculatus* bites in a severe reactor.

B. Histopathology of late reaction to *Anopheles quadrimaculatus* showing severity and extent of reaction, hematoxylin-eosin, ×90.

The *Pediculus humanus* represents an arthropod which feeds longer than a mosquito. The few biopsies available show that in the nonsensitive individual the reaction to the bite of the *Pediculus* is not as extensive as is the reaction to the mosquito. It appears, however, that the same general patterns of the immediate and late reactions are followed. In spite of the longer duration of feeding the sections available of the immediate area of the bite reaction do not show large numbers of red cells. The changes in the epidermis are also not prominent. Biopsies of bites from pediculi in persons with no visible clinical reactions revealed a small perivascular infiltrate which even in the earliest phases was

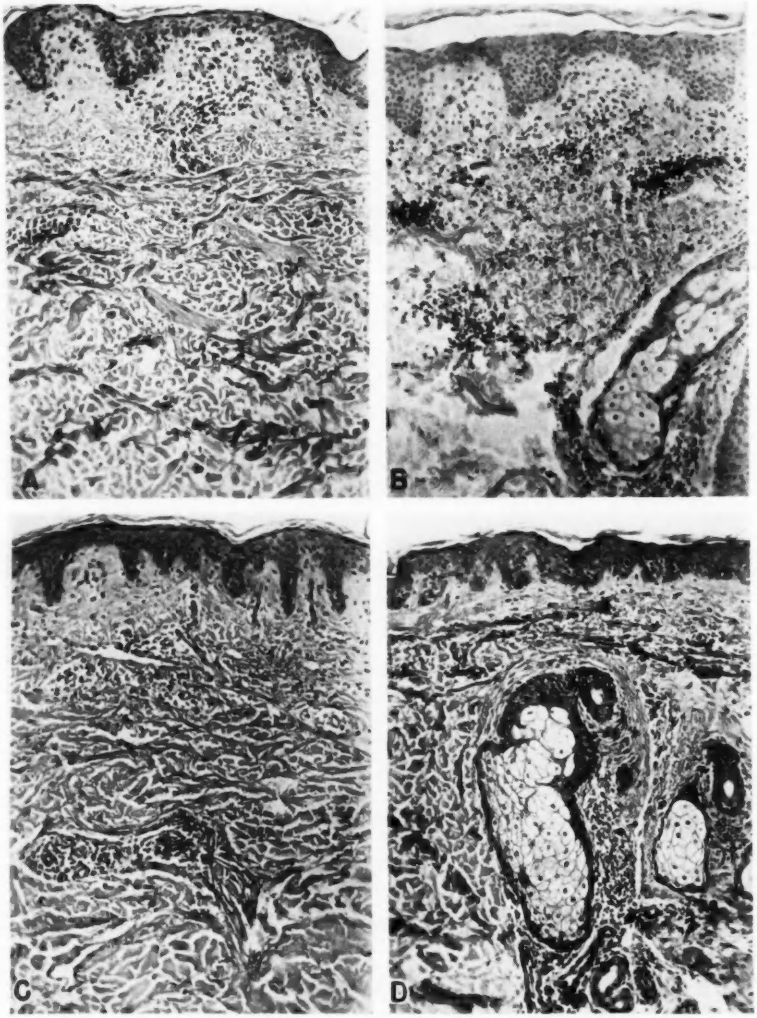

FIG. 3. Progressive development of reaction to *Aedes aegypti* in a moderate reactor, hematoxylin-eosin, ×160.

A. 30 minute reaction showing edema and perivascular infiltrate.

B. 6 hour reaction showing the height of the reaction of the acute phase with an increased number of polymorphonuclear leukocytes and spread of the infiltrate.

C. 2 day reaction showing a decreased intensity.

D. 5 day reaction showing a further decrease in intensity with no edema but persistence of reaction about adnexa.

composed of lymphocytes with occasional polymorphonuclear leukocytes and eosinophiles. The eosinophiles and polymorphonuclear leukocytes disappeared in two days. Biopsies of *Pediculus* bites in moderately sensitive individuals were similar to biopsies of mosquito bites.

Because the biopsies of the flea bites in our series did not include sensitive individuals, material was obtained from Lunsford and Templeton of Oakland, California (1949). Their material revealed the same general pattern of extensive cellular response in sensitive individuals that was seen in the mosquito and *Pediculus* bites. Biopsies in our non-sensitive individuals showed essentially the same progression as *Pediculus* bites. It is felt that our difficulties of finding persons who react to fleas and lice and persons who do not react to mosquitoes is due to the frequency of exposure to mosquitoes and the lack of frequent exposure to fleas and lice in our community. Exposure is of course necessary for sensitization. At present, incomplete studies of bites of *Cimex lectularius* also show no great deviation from the pattern produced by the mosquito.

Bites produced by the *Eutrombicula alfreddugesii* were characterized by severe epidermal changes with marked intraepidermal edema with central bullous formation. The patterns of the edema collection in the epidermis seemed to suggest the hypostome arrangement as indicated by the textbooks of entomology (Matheson, 1950). However, no biopsies have been done as yet with the chigger *in situ* and it is not possible to determine in the few studies done whether this actually does indicate such a feeding pattern. Pseudoepitheliomatous hyperplasia has not been seen in the section of chigger bites which are available to us. A heavy perivascular infiltrate was also observed. The tremendous intradermal cellular response to the chigger even after a period of some days demonstrates how long the clinical type of late nodular response can persist.

A four day old reaction to *Amblyomma americanum* revealed moderate intraepidermal edema with a small intraepidermal vesicle. The dermis shows marked edema with a prominent perivascular inflammatory infiltrate of lymphocytes and large numbers of histiocytes. In one biopsy four hours after feeding, bits of "cuticular" material were observed in the upper dermis. This material was observed more clearly with polarized light.

One phase of this study included an attempt to obtain sections of skin with the biting parts of various arthropods *in situ*. This procedure involves technical difficulties due to the difference in density among other factors, of skin and cuticle. However, a small number of adequate sections have been obtained. Our sections of the fascicle of the *Anopheles quadrimaculatus* and of the *Aedes aegypti* do not indicate whether the mosquito feeds by the intravascular technic or by pool feeding. Intravascular feeding is suggested in some instances by the absence of tissue hemorrhage and by the presence of the fasicle close to blood vessels. In other sections, blood cells are seen in the tissue in the neighborhood of the fasicle suggesting indirectly the possibility of pool feeding. Biopsies with the mosquito proboscis *in situ* demonstrate how deeply the fascicle can penetrate into the mid dermis of the human skin. Sections of the *Pediculus humanus* were observed on but not in the skin.

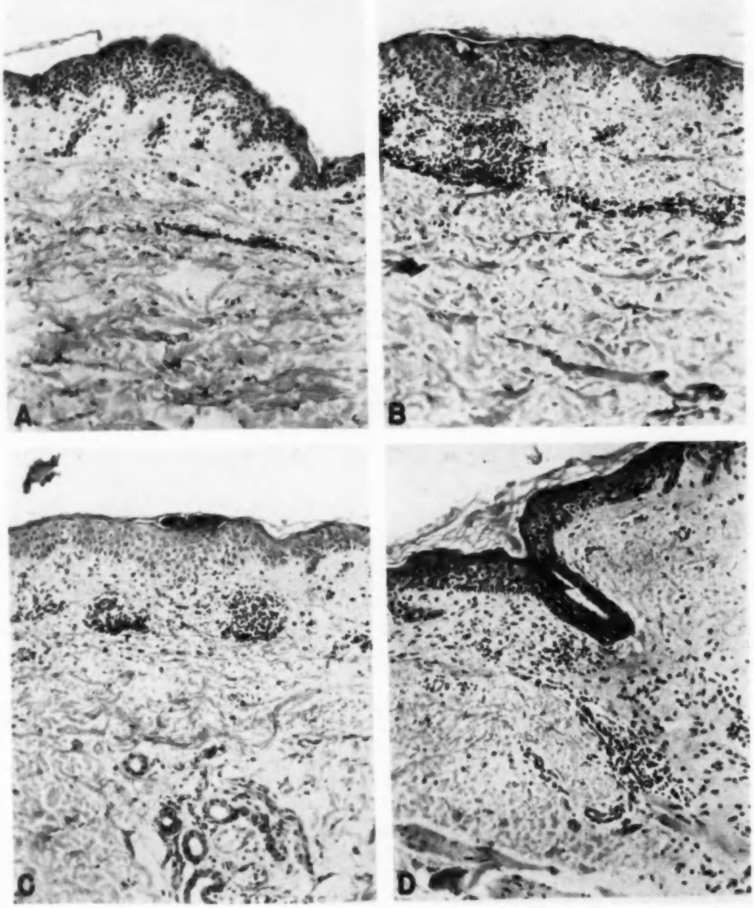

FIG. 4. Progressive development of reaction to *Pediculus humanus* in a clinical nonreactor, hematoxylin-eosin. ×160.

A. 5 hour reaction showing slight edema and sparse inflammatory infiltrate.

B. 1 day reaction showing spongiosis, edema in the dermis, and localized intense cellular infiltrate.

C. 2 day reaction showing essentially same changes with a superficial vesicle.

D. 4 day reaction showing persistence of moderate edema but decreased inflammatory infiltrate.

The study of the arthropod or its biting parts *in situ* help to evaluate the influence on the biting reaction of retained portions of the arthropod in the tissue. Allen (1948) believes this to be a significant factor in the development of chronic reactions to insect bites.

Two general types of chronic insect bite reactions have been described: the intrinsic nodular response and the neurodermatitic response secondary to scratching. Histologically the intrinsic nodular response consists of a dense polymorphous, perivascular and perifollicular inflammatory reaction composed of lymphocytes, histiocytes, eosinophiles and plasma cells. Secondary lymphoid follicles with germinal centers may be present. This is the type of lesion which may

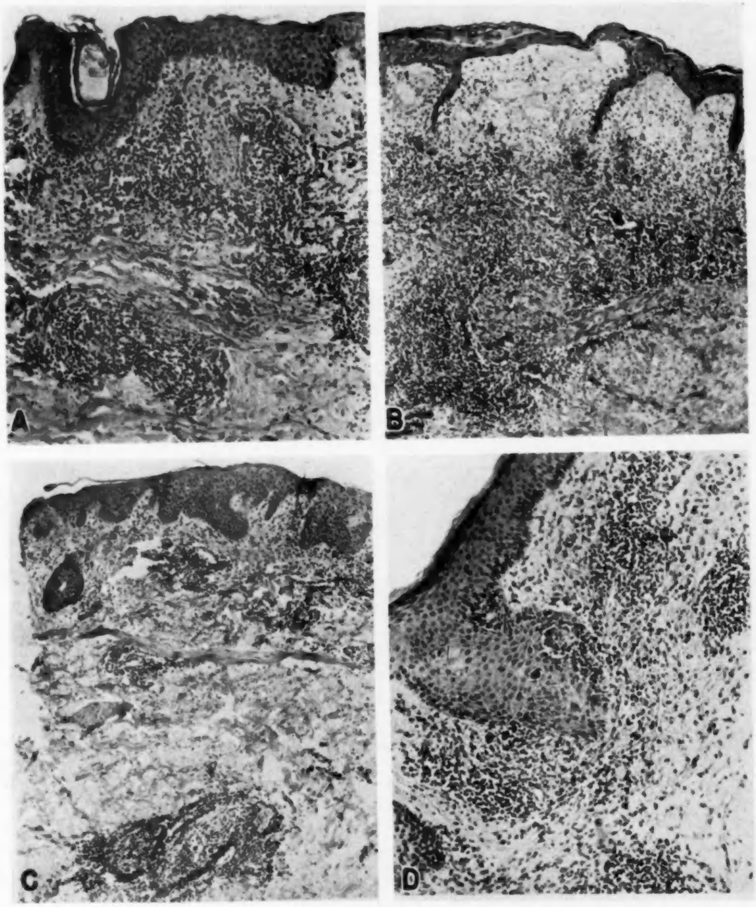

FIG. 5. Late severe reactions to different arthropods showing general similarity.
A. 3 day reaction to *Aedes aegypti*, hematoxylin-eosin, ×160.
B. 4½ day reaction to *Eutrombicula alfreddugesii*, hematoxylin-eosin, ×90.
C. 4 day reaction to *Pediculus humanus*, hematoxylin-eosin, ×90.
D. 4 day reaction to *Amblyomma americanum*, hematoxylin-eosin, ×160.

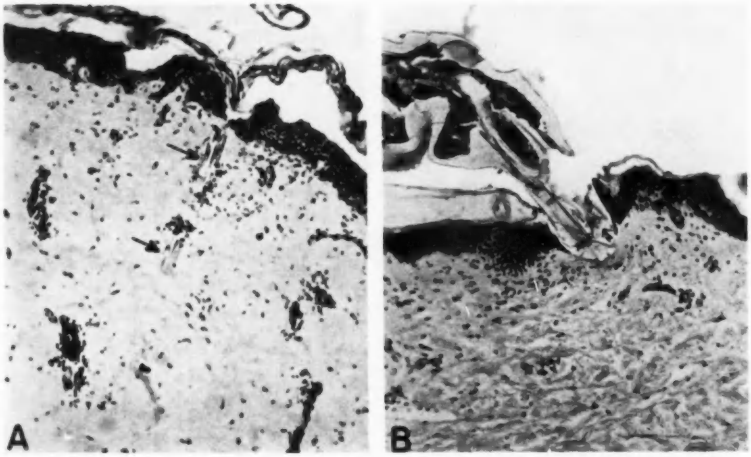

FIG. 6. Biting parts of arthropods in situ.
A. *Aedes aegypti* while feeding, hematoxylin-eosin, ×160.
B. *Amblyomma americanum* while feeding, hematoxylin-eosin, ×160.

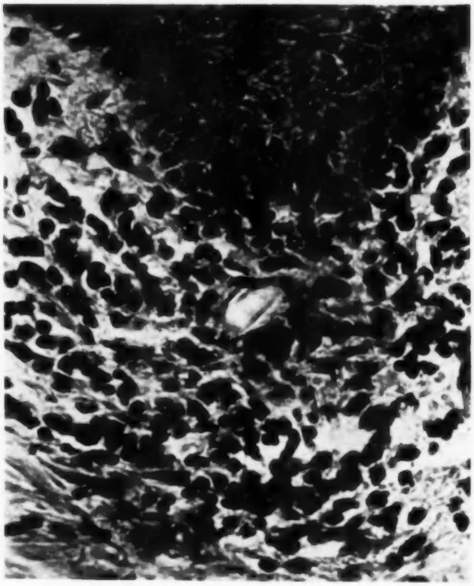

FIG. 7. Retention of fragments of *Amblyomma americanum* 4 hours after feeding, polarized light, hematoxylin-eosin, ×650.

simulate the so-called benign cutaneous lymphocytoma. We have not, as yet, encountered this lesion. The neurodermatitic response, which is more common in our experience, can be recognized clinically by the presence of a lichenified or thickened skin usually spread over an area considerably larger than the primary site of bite reaction. Microscopically the picture is no different from the neurodermatitic reaction which may be seen in response to any type of local scratching phenomena in response to persistent itching. There is a definite regular or irregular acanthosis, a perivascular lymphocytic infiltrate confined chiefly to the upper layers of the dermis, and occasionally, some diffuse fibrosis scattered through the dermis. Clinically, we have not, as yet, encountered the granuloma pyogenicum type of reaction to insect bites.

Preliminary studies have been done with *Aedes aegypti* and *Anopheles quadrimaculatus* biting over areas of skin injected previously with antihistamines, cortisone, compound F[2], and suitable controls including the active vehicles of these steroids. The inflammatory reaction to the steroid and its vehicle renders interpretation of the reaction to the superimposed mosquito bite difficult. Clinical studies so far suggest no inhibition by local cortisone but definite inhibition by local compound F (Goldman, in press). This local reaction by Compound F may persist for some months after the local injection.

With Dr. Eugene Saenger, Director of the Radio Isotope Laboratory, Cincinnati General Hospital, preliminary studies have been done with isotopic technics employing radioactive phosphorus, P^{32}, and iodine, I^{131} for feeding to mosquitoes, ticks, and bedbugs. The mosquitoes fed on sugar solutions containing P^{32} and I^{131}. The larger arthropods did not feed but were injected. However, they did not bite following injection of the isotope. The dosage varied for the mosquitoes from 100 microcuries to 3 millicuries, the latter dose apparently lethal with this feeding technic. Radioactivity is transmitted to the skin following the bite of the mosquitoes. Autoradiographs now available, however, do not permit determination of the pattern of the insect bite.

SUMMARY AND DISCUSSION

The morphology exhibited in the sections of arthropod bite reactions permits a dynamic interpretation. In the search for the blood meal, the biting mechanism of the arthropod appears to penetrate through the epidermis with minimal difficulty. Some spreading factor in the saliva is suggested by the rapid spread of the tissue reaction. The perivascular phases of the reaction also suggest a rapid vascular transportation of the material injected. The sensitivity of an individual to the bite of a given arthropod influences the intensity of the inflammatory response. However, an individual may be sensitive to one arthropod and insensitive to the bites of others. The insensitive individual who develops no clinical bite reaction may be bitten without being aware of it. The histological studies of Boltz (1951) of the skin reactions following injections of needle puncture show that the changes in the skin following a bite by an arthropod is not one of simple

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[2] 11-Dihydro-17-Hydroxycorticosterone-21-Acetate Merck and 17-Hydroxycorticosterone-21-Acetate (Compound F acetate) furnished by Dr. Augustus Gibson, Merck & Co.

"mechanical" puncture even in a non-reactive individual. The persistent late nodular response may be due to the intrinsic nature of the bite (including retained cuticular fragments?) or to a neurodermatitic response secondary to the scratch reaction to itching.

Histopathologic methods are valuable in the study of bite reactions. Improvements such as vacuum freeze drying technics and histochemical studies may permit more detailed studies of the variations in the cellular response of the individual to the bites of arthropods. With better fixation technics it may be possible to pick up fine bodies retained after the insect bite. Improvement in the technic of study of the biting parts *in situ*, will make possible observations relating to the effect of the artificially induced retention of a piece of the biting apparatus within the skin. It is also possible that isotopic studies with other isotopes and more refined autoradiographic technics may enable us to follow the path of the bite more accurately. Progress in the study of the peripheral mechanisms of reaction of Compound F deposited locally may also help in the analysis of the bite reaction.

With the bite reaction of an individual determined, it should then be possible to study in detail local and systemic factors which may modify some parts of the bite reaction, as for example, the vascular response, the inflammatory cell response, the degree of pruritus, additive local trauma, etc.

CONCLUSIONS

Histopathology of the arthropod bite reaction is a valuable tool in the study of the mechanism of this reaction. In general there is a great degree of basic similarity among the immediate and late reactions to the bites of various arthropods. The intensity of the bite reaction appears to be severer following bites of chiggers and ticks than the insects and varies according to the sensitivity of the individual. The characteristic response of the hypersensitive individual is an increased inflammatory reaction with a greater predominance of eosinophiles within the inflammatory infiltrate. Studies with portions of the biting apparatus *in situ*, autoradiographs, and the effect of local cortisone and compound F were not sufficiently complete to warrant any definite conclusions. Local cortisone had no effect but Compound F locally applied inhibited the bite reaction.

REFERENCES

ALLEN, A. C. 1948, Persistent "insect bites" (dermal eosinophilic granulomas) stimulating lymphoblastomas, histiocytes, and squamous cell carcinomas, *American Journal of Pathology* **24**: 367–387.

ASH, J. E. AND SPITZ, S. 1945, Pathology of Tropical Diseases, W. B. Saunders Co., Philadelphia and London, p. 325.

BOLTZ, W. 1951, Histologische Untersuchungen an Injektionsstichspuren, *Deutsche Ztschr f.d. ges gerichtl Med.* **40**: 181–187.

BROWN, A., GRIFFITHS, T. H. D., ERWIN, S. AND DYRENFORTH, L. Y. 1938, Arthus' Phenomenon from mosquito bites, *South. Med. J.* **31**: 590–596.

GOLDMAN, LEON: A controlled study of the local effect of 17-hydroxy-corticosterone-21-acetate (Compound F) on the severe reactions to mosquito bites, *J. A. M. A.* (In press).

GOLDMAN, LEON AND JOHNSON, PATRICIA AND RAMSEY, JAMES: The insect bite reaction. I. The mechanism, *J. Invest. Dermat.* (In press).

GORDON, R. M. AND CREWE, W. 1948, The mechanisms by which mosquitoes and tsetse-flies obtain their blood meal, the histology of the lesions produced, and the subsequent reactions of the mammalian host, *Annals of Tropical Medicine and Parasitology* **42:** 341–344.

LUNSFORD, C. J. 1949, Flea problem in California, *Arch. Dermat. & Syph.* **60:** 1184–1202.

LUNSFORD, C. J. AND TEMPLETON, H. J. Personal Communications to the Authors.

MATHESON, ROBERT 1950, Medical Entomology 2nd Edition, p. 108, Comstock Publishing Co., Inc., Ithaca, New York.

ROCKWELL, EVELYN M. AND JOHNSON, PATRICIA: The insect bite reaction. II. Evaluation of the allergic reaction, *J. Invest. Dermat.* (In press).

TRAGER, WM. 1939, Acquired immunity to ticks, *J. Parasitology* **25:** 57–81.

WINER, L. H., STRAKOSCH, E. A. 1941, Tick bites—*Dermacentor variabilis* (Say), *Journal Investigative Dermatology* **4:** 249–258.

MINUTES OF THE 34TH ANNUAL MEETING OF THE NATIONAL MALARIA SOCIETY[1]

OFFICERS

President—Dr. Justin M. Andrews, Atlanta, Georgia
President-Elect—Mr. W. H. W. Komp, College Park, Maryland
Vice-President—Mr. H. W. Van Hovenberg, Mt. Pleasant, Texas
Secretary-Treasurer—Dr. S. W. Simmons, Savannah, Georgia

DIRECTORS

Dr. Lloyd E. Rozeboom, Baltimore, Maryland
Dr. George H. Bradley, Atlanta, Georgia
Dr. Paul F. Russell, Rome, Italy—*Past President*

Thursday, November 15, 1951

The National Malaria Society convened for its 34th annual meeting at 8:30 A.M., in the Gold Room of the Congress Hotel in Chicago, Illinois, with President Justin M. Andrews presiding. Ten papers were given, followed by the President's address, the presentation of the Joseph Augustin LePrince Award to Mr. J. A. LePrince, and the business session.

Friday, November 16, 1951

The scientific session met at 2:00 P.M. in the Casino Room of the Congress Hotel to hear a Panel on "Resistance of Insects to Insecticides," followed by other scientific papers. After opening the session, President Justin M. Andrews turned the chair over to Dr. S. W. Simmons, Moderator of the Panel. Four talks were given on the Panel subject by as many experts in this field. They were Dr. A. D. Hess, Dr. E. F. Knipling, Dr. George C. Decker, and Dr. Leigh E. Chadwick. Following conclusion of the Panel, five additional papers were presented; three papers had previously been withdrawn from the program.

Saturday, November 17, 1951

The National Malaria Society reconvened at 2:00 P.M. for a conjoint meeting with the American Society of Tropical Medicine and the American Society of Parasitologists. The meeting was opened by Dr. Paul F. Russell, President of the American Society of Tropical Medicine. After the presentation of several papers, the chair was turned over to Dr. Justin M. Andrews, and later to Dr. Eloise Cram. A program of twelve papers was presented. The meeting adjourned at 5:00 P.M.

MINUTES OF THE BUSINESS MEETING

November 15, 1951

The minutes of the 1950 annual meeting in Savannah, Georgia, were approved as published in the Journal of the National Malaria Society in March 1951. The Secretary-Treasurer reported as follows:

Of the 1950 roster of 516 active members (527 reported at 1950 annual meeting, minus 11 shown on 1950 report as being suspended for lack of address), five (Dr. E. L. Bishop, Vice-President of the Society, Mr. Wade Hampton Bolton, Colonel Charles F. Craig, Dr. Alberto Missiroli, and Mr. N. Krishna Murthy) have been lost by death, four have resigned, and 32 have been dropped because of delinquency in dues. During the year 44 new members have been elected, making an active membership of 519. This represents an increase of three members. Of the membership, 417 were in good standing on October 31, 1951.

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[1] Held conjointly with the American Society of Tropical Medicine, the American Academy of Tropical Medicine, and the American Society of Parasitologists, Chicago, Illinois, November 15, 1951.

The status of the treasury at the close of business October 31, 1951 was:

Balance reported—October 31, 1950	$5,527.67
Receipts from delinquent, current, and advance dues, subscriptions, sales of back issues, sales of Symposium Issue, advertising, interest, and miscellaneous	7,597.19
Total	$13,124.86
Expenditures before paying for the fourth issue of the 1951 Journal, but including the cost of the fourth issue of the 1950 Journal	6,171.04
Balance on hand October 31, 1951	$6,953.82

Of the balance on hand October 31, 1951, $5,607.75 is in the Publication Fund, and $1,346.07 is in the Operating Fund.

Dr. A. D. Hess reported for the Committee on Auditing, and stated that the books and accounts of the Secretary-Treasurer had been examined and found in order. The Society adopted the report of the Secretary-Treasurer and the Auditing Committee.

The report of the meeting of the Board of Directors on November 14, 1951 was read and accepted. The minutes of this meeting are printed elsewhere in the Journal.

Dr. Griffith E. Quinby gave a brief report for the Committee on Statistics showing some interesting illustrative material of malaria incidence.

Dr. George H. Bradley reported that the Committee on Lectureship and Award had been successful in securing a gift of $500.00 to be used for the LePrince Award in 1951.

Dr. A. D. Hess gave a resume of advertising in the Journal of the National Malaria Society during 1951. It showed approximately twice as many advertisers as in 1950.

Dr. Justin M. Andrews, Chairman of the Committee on Amalgamation, gave a resume of the activities of this Committee during the year.

For the Committee on Resolutions, Dr. Lloyd E. Rozeboom introduced resolutions expressing the Society's profound regret upon the death of Dr. E. L. Bishop, Mr. Wade Hampton Bolton, Colonel Charles F. Craig, Dr. Alberto Missiroli, and Mr. N. Krishna Murthy, and instructed the Secretary to communicate an expression of sympathy to the families concerned. A resolution was adopted acknowledging the generosity of the Michigan Chemical Corporation in providing a $500.00 honorarium to accompany the presentation of the Joseph Augustin LePrince Award of the National Malaria Society. The Secretary was instructed to express the Society's sincere gratitude to the Michigan Chemical Corporation and to Mr. R. N. Wismer, its President, for providing this honorarium. Other resolutions conveyed the appreciation of the Society to the Committee on Local Arrangements; to the several host organizations consisting of the University of Chicago, Roosevelt College, the University of Illinois Medical School, Northwestern University, and the Chicago Medical School; to the commercial companies sponsoring the hospitality sessions, and the ladies' entertainment program. The Secretary was instructed to express to each of the above the sincere thanks of the Society.

The Tallying Committee reported that as a result of the votes counted, the following officers were elected for 1952:

President-Elect—Dr. Martin D. Young
Vice President—Dr. A. D. Hess
Director for 3 years—Dr. A. J. Walker

With reference to amalgamation of the National Malaria Society and the American Society of Tropical Medicine, Dr. Paul F. Russell presented the following resolution: Mr. President, I move that, since the objective of the National Malaria Society has been attained through the elimination of malaria as a major public health problem in the United States, the National Malaria Society, upon the formation of the American Society of Tropical Medicine and Hygiene shall transfer its total assets, liabilities, and membership to the new Society, and by this action shall dissolve itself. This resolution was unanimously adopted by the Society.

It was recognized that the National Malaria Society would cease to exist after the formation of the American Society of Tropical Medicine and Hygiene, therefore, the Secretary-Treasurer and the Editor of the National Malaria Society were authorized, at the Business Session, to continue their present official duties in completing the business of the Society up to the time it was taken over by the Secretary-Treasurer and the Editor of the new Society.

The Secretary-Treasurer discussed the invitations which had been received from the Medical Branch of the University of Texas at Galveston for holding meetings in that city, and called attention to the fact that the Board of Directors had recommended that the Secretary express a strong preference for holding the 1952 meeting in Galveston, Texas, where the Medical Branch of the University of Texas had offered to act as host. The Society authorized the Secretary to act for it in choosing the 1952 meeting place.

The business meeting adjourned sine die at 9:20 P.M.

MINUTES OF THE MEETING OF THE BOARD OF DIRECTORS NATIONAL MALARIA SOCIETY[1]

The Board of Directors of the National Malaria Society met at 4:20 P.M., November 14, 1951, in Parlor G of the Congress Hotel, Chicago, Illinois. Members present were Dr. Justin M. Andrews, President; Mr. H. W. Van Hovenberg, Vice-President; Dr. Paul F. Russell, Director; Dr. George H. Bradley, Director; Dr. Samuel W. Simmons, Secretary-Treasurer; and Dr. G. Robert Coatney, Editor.

The actions of the Board of Directors during 1951 were reviewed as follows: approved committee appointments made by President Andrews; approved contribution of $50.00 toward a bust of General Gorgas in the New York University Hall of Fame; and elected 44 members for 1951.

The report of the Secretary-Treasurer was accepted, and Dr. S. W. Simmons received the congratulations of the Board of Directors for the way in which the business had been handled during the past year.

The report of the Editorial Board was accepted, and Dr. G. Robert Coatney was commended for the excellent manner in which he had fulfilled the duties of Editor. The Directors authorized the printing of 100 pages per quarterly issue in the 1952 Journal.

The report of the Committee on Lectureship and Award, by Dr. G. H. Bradley, stated that $500.00 had been contributed by the Michigan Chemical Corporation for the J. A. LePrince Award for 1951. Dr. Bradley was congratulated for his efforts and success in accomplishing the difficult task of securing the contribution.

The report of the Committee on Amalgamation was given by the Chairman of that Committee, Dr. Justin M. Andrews. The actions of the committee at both the meeting in Boston in May 1951 and the meeting in Chicago on November 14, 1951 were reviewed and accepted by the Board.

The Directors reappointed the members of the Amalgamation Committee and authorized them to act for the Society, as indicated, in completing the business of amalgamation. The Committee was also authorized to act, in conjunction with members of the Amalgamation Committee of the American Society of Tropical Medicine, as a Nominations Committee to select a suggested slate of officers for the proposed American Society of Tropical Medicine and Hygiene.

The Board of Directors recommended that the funds which are turned over to the new American Society of Tropical Medicine and Hygiene be used as necessary to defray the expenses of the Joseph Augustin LePrince Award.

Dr. Justin M. Andrews appointed committees to canvass the ballots for election of officers and for amalgamation, to audit the books, and to draw up appropriate resolutions.

Dr. G. Robert Coatney was reappointed as representative to the A.A.A.S. Council.

The Board of Directors reappointed Dr. S. W. Simmons as Secretary-Treasurer which he accepted until the completion of amalgamation, to be effected at the 34th annual meeting and completed early in 1952. The Directors also authorized the Secretary to continue a $5,000.00 bond during his tenure in 1952. Approval was granted for the Secretary to handle all routine business for the Society.

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[1] **Wednesday, November 14, 1951, Congress Hotel, Chicago, Illinois.**

The Secretary was instructed to express a strong preference for holding the 1952 meeting in Galveston, Texas, where the Medical Branch of the University of Texas has offered to act as host. This invitation was extended for the 1951 meeting and again for 1952.

The Directors approved honoraria in the amount of $300.00 for the Secretary's stenographer, up to $50.00 for the Editor's stenographer, and up to $35.00 for the Secretary for expenses incurred relative to attendance at the meeting. Two hundred dollars, or as much thereof as needed, was approved for stenographic assistance for that portion of 1952 during which the Secretary may handle the affairs of the Society, prior to completion of amalgamation.

The meeting adjourned sine die at 6:15 P.M.

BOOKS RECEIVED

Note: Books received for editorial consideration will be intermittently listed. This acknowledgment must be regarded as an adequate expression of appreciation for the courtesy of the author or publisher. Selections will be made for review in the interest of our readers.

Malariologia Prática; Preparado sob os Auspicios de Divisão de Ciencias Médicas do Conselho Nacional de Pesquisas dos E.U.A. Por Paul F. Russell, Luther S. West, e Reginald D. Manwell. Traduzido da la Edicão em Ingles, de W. B. Saunders Co., Filadelfia e Londres, 1946. 810 pp., 238 illustrations, 8 in color. Rio de Janeiro; publicado pelo Serviço Especial de Saúde Pública, 1951.

Esquistosomiase Mansoni Hepato-Esplênica. Tese de concurso para Catedrático de Clínica de Doencas Tropicais e Infectuosas da Faculdade de Medicina, S. Paulo, by João Alves Meira. 607 pp., illustrated. São Paulo, Brasil. Published by the author. 1951.

The Zoology of Tapeworms. By Robert A. Wardle and James Archie McLeod, University of Manitoba, Winnipeg, Canada. 780 pp., with 419 illustrations. Minneapolis: Published for the University of Manitoba by the University of Minnesota Press, 1952. Price $12.50.

Infective Hepatitis. British Medical Research Council Special Report Series No. 273. Stud. in East Anglia during the period 1943–47, by F. O. MacCallum, A. M. McFarlan, J. A. R. Miles, M. R. Pollock and C. Wilson. 144 pp., paper. London, His Majesty's Stationery Office, 1951. Price 4/6.

Illustrated Map of the Anopheline Imagines of Indonesia (English translation). Published by the Ministry of Health of Indonesia (Section: Malaria Control). 2nd edition, corrected and supplemented, with 16 pages of figures illustrating 72 species. Djakarta, December, 1949.

Textbook of Clinical Parasitology. By David L. Belding, M.D., Professor of Bacteriology and Experimental Pathology, Emeritus, Boston University School of Medicine. 2d edition: 1139 pp., illustrated. New York, Appleton-Century-Crofts, Inc., 1952. Price $12.00.

Medical Certification of Cause of Death. Instructions for Physicians on Use of International form of Medical Certificate of Cause of Death. Bulletin of the World Health Organization, Supplement 3. Available also in French. 20 pp., paper. Published by the W. H. O., Palais des Nations, Geneva, 1952. Price 0.20, 1/-, Sw fr. 0.75.

Histopathological Technic, Including a Discussion of Botanical Microtechnic. By Aram A. Krajian, ScD. and R. B. H. Gradwohl, M.D. 2d edition: 362 pp., with 131 illustrations, 7 in color. St. Louis, The C. V. Mosby Company, 1952. Price $6.75.

BOOK REVIEWS

Rosenau's Preventive Medicine and Hygiene, by KENNETH F. MAXCY, Professor of Epidemiology, Johns Hopkins University School of Hygiene and Public Health, with 26 Contributors. 7th edition. 1462 pp., 131 illustrations, frontispiece in color. New York: Appleton-Century-Crofts, Inc., 1951. $14.00.

The appearance of a new edition of "Rosenau" is an important and rather exciting event in the world of Public Health. We have been waiting fifteen years for this, the seventh edition, and few will be disappointed in it. The striking advances in the techniques, strategy and principles of modern health practice are competently reflected in a text which has been almost completely rewritten by Dr. Maxcy and his team, to whom Rosenau, toward the end of his life, wisely confided the task of revision.

It is obvious that some effort was loyally made to keep as much of the old version as possible, and some curious compromises are to be found between the old and the new, but remarkable changes not only in the content of our knowledge but in our point of view compelled a reconstruction rather than a revision.

The strength of the old "Rosenau" was in the communicable diseases and sanitation, and there has been a shift of emphasis here. The word "disinfection" does not appear in the new index, while a whole section on epidemiology and "statistical reasoning" has grown out of what, in "Rosenau," was hardly more than a paragraph in the description of a disease. Having dominated the pestilences which decimated our fathers, we have established a sort of *pax Romana* with our enemies which affords an unprecedented opportunity to cultivate the arts of healthful living. Nevertheless, smallpox opens the first chapter as it used to and takes over the frontispiece, a sign of the precariousness of our peace, maintained only by ceaseless preventive activity. So, while we are apt nowadays to disparage the importance of the communicable diseases, Maxcy still prudently gives them 518 pages to Rosenau's 308; and although most of the dreaded ones have lost their terror—typhoid, malaria, diphtheria, the infant diarrheas, scarlet fever, syphilis and to some extent tuberculosis—there is still a sizable collection of riddles gathered in a chapter which Rosenau labeled "miscellaneous," but which are now more logically grouped under the heading "unclassified as to mode of spread."

The chapters which have changed the least are those on sewage and garbage disposal, with much the same text, figures and tables as before. Apparently house plumbing and the composition of American sewage have not changed much in fifteen years, and this is understandable because sanitation is one of the oldest branches of health practice. Furthermore, it is a technical field with a vast literature of its own and can be given only superficial treatment in a book of this kind. Nevertheless, it is becoming obvious that the sanitary engineer of the future will have to devote his peculiar talents more and more to the economical utilization, rather than the mere safe disposal of the enormous variety and quantity of waste material which human beings and particularly Americans in their careless exuberance now throw away. This has received no special emphasis in the present volume.

The table of the incidence and mortality of typhoid fever has, of course, had to be drastically revised downwards since 1935—in the United States, that is; not, unfortunately, in less favored environments. This points up another difference between the two editions. The present book is less a *vade mecum* than the old encyclopedic "Rosenau" which was a meticulous guide to all technical procedures and a bible to early public health workers throughout the world. Indeed the old book has been in constant demand abroad up to the present time.

To keep up with the newer trends and problems at home, the book has had to sacrifice in some measure its appeal to those faced with primitive situations. It is rumored that a new edition of General Dunham's excellent book on "Military Preventive Medicine" is in preparation which should go far to fill this need.

The great distinction of the new edition is its positive approach to health problems. In modern fashion, it stresses the promotion of health in childhood and old age, not merely our defenses against disease. Infant and maternal mortality (ten pages in "Rosenau") is transformed into the maintenance of maternal and child health (eighty-six pages in "Maxcy") and the chapter on vital statistics becomes "The Health of the Population." Public health organization and activities are adequately handled, together with a chapter on international public health and its various agencies.

Certain omissions were doubtless unavoidable, if only to keep down the size of the book; it weighs five pounds as it is. Sections in the old "Rosenau" entitled Vision, Contraception, Heredity and Genetics, Drug Addiction, Soil, Disinfection and others are not to be found in the index of the new edition. They will not be missed; they belong in more specialized textbooks. Two broad subjects however, on the expanding frontier of public health, are virtually ignored or receive at best a very conservative treatment. One is the highly controversial field of medical care which is being threatened with invasion by the cohorts of Greater Public Health, and the other is the importunate population problem, for which public health is often held responsible and, as the most efficient operating agency of applied social science, is expected to provide a solution. The next edition, which we hope may follow at a shorter interval, will find a philosophical consideration of such issues unavoidable.

All in all, this new book reflects the advancement in our public health and is better than the old in content and arrangement. It faces new problems with sound knowledge, a wide shift in emphasis and a modern approach. It will be indispensable to the student and practitioner of public health, and can be used in teaching medical students. Rosenau said in his first preface, forty years ago, "No other book covers the broad field considered in this volume." That is still true.

L. W. HACKETT

Pharmacopoea Internationalis. Vol. I, 1st edition, pp. XVIII plus 406., World Health Organization, Geneva, Switzerland, 1951. 35/–, $5.00, Sw. fr. 20.

To understand the Pharmacopoea Internationalis, one must realize that it is a mystery story in disguise. Fifty years ago the first convention met to formulate an international pharmacopoeia. International nomenclature and standards were obviously needed for communication between physicians and investigators in different countries. An international nomenclature had been achieved without difficulty in sciences that varied from botany to astronomy. Why then, was half a century required to produce a book a third as large as the United States Pharmacopeia? Committee meetings are notoriously wasteful of time, but fifty years is preposterously long, even for a committee meeting!

Careful examination reveals that the Pharmacopoea Internationalis is an exciting palimpsest. Behind the blandly objective text one may discern the imperfectly erased history of violent battles. The play of cloak-and-dagger arises from the very nature of the sciences of pharmacy and pharmacology. Various drugs are property. They are protected by patents or copyrights. Decisions by the international convention could easily give a practical monopoly to one or another company. Hence, all disputes had a vitriolic quality that would be unthinkable, for example, in a science like astronomy. The stars are not private property. No one can patent or copyright them. Whether a certain nova is included in an official list, and how it is named, is not a matter of millions of dollars of profit or loss to any corporation.

The choice of drugs is the primary target as far as commercial competition is concerned. It is easy for the pharmacopoeia to include venerable antiques, but valuable recent drugs are likely to bring the international convention to an impasse. One may suspect that this reason, more than the difficulty in standardization, has caused all of the antibiotics to be deferred to a later volume.

The selection of drugs is most complete and up-to-date for the group used in tropical diseases. This results from a grave problem which has afflicted tropical countries during the past five years. Irresponsible drug companies, which do not have a respected name to protect, have sold large amounts of substandard drugs in the lands between the Tropic of Cancer and the Tropic of Capricorn.

Nomenclature was a second and continuous cause of battle. In the United States a patent on a drug expires at the end of seventeen years. A copyright on a name, however, may be renewed every twenty years, and for practical purposes, a name may create a permanent monopoly. The proprietary name will enjoy this advantage if it is adopted as official, or if the official name is sufficiently awkward as to be unusable.

The problem of specific names is serious enough, but the decision to state all official titles in Latin brought further trouble. It seems logical to use Latin on the precedent of Linnaean botanical and zoological terminology. Linnaeus made his classification of plants and animals, however, at a time when Latin still had valid claims as the international language of learning. This is no longer true. The B.N.A. anatomical terminology, which was formulated in 1895, did not simplify anatomical nomenclature. Rather it created one more system of nomenclature, with the result that anatomists and surgeons in our own country speak different languages. Dubious, indeed, is the advantage to modern pharmacology of attaching a Latin case-ending to a familiar word and calling the result "Latin". Caesar and Cicero, as well as Celsus and Galen, would have denied that Acetarsolum or Ouabainum or Sulfadiazinum are Latin words. The predominance of English in recent international conferences suggests that scholars throughout the world could more easily recognize English words without artificial Latin case-endings.

A third feud arose from the dogma that, except in a few cases where international units are needed, all doses must be expressed in decimal parts of a gram. The failure to use milligrams and micrograms requires the statement of dosage of many drugs to be expressed in the fifth decimal place. Descriptions of standards are usually carried to the seventh decimal place, and sometimes even to the ninth. One must hope that pharmacists are more accurate in dealing with decimal fractions than physicians, since the Pharmacopoea Internationalis gives the dose for internal administration of some drugs which are best employed in detective stories. They include aconite, arsenic trioxide, bichloride of mercury, and strychnine.

Despite these handicaps, the Ph.I. has many advantages over national pharmacopoeias. The U.S.P. has been made the law of the land, hence, it must be so exact that no scoundrel can twist its meaning in court. The Ph.I., however, needs merely to be clear enough for an honest and reasonable man to understand. Thus, it defines the word "negligible" as "a quantity not exceeding .0005 g." The dosage stated for numerous drugs is one-half to one-fifth of this amount and, therefore, is "negligible" by definition. No honest and reasonable man, however, would misunderstand the intent and use of the definition, so, a more elaborate statement is unnecessary. Since the Ph.I. assumes that its use will be in the pharmacy and laboratory rather than in courts of law, abbreviations are used freely. Likewise, descriptions are more simple and brief than the U.S.P. would dare to trust in the hands of an attorney who might wish to quote some sentence apart from its context in a damage suit.

The book has an appendix stating the usual mode of administration of each drug, the usual single and daily doses, and also the maximal safe single and daily doses. Despite the four drugs which I have mentioned as chiefly of homicidal interest, the Ph.I. does not have the usual load of museum relics, but contains a group of drugs adequate for practice on a desert island. It finds no need for including items which are not used for their pharmacologic effect (such as adhesive plaster, surgical sutures, and gauze bandages). Nor does it need to standardize four kinds of water for various medical purposes, as does the 1950 U.S.P.

The Pharmacopoea Internationalis is clearly an advance toward defining an international medical vocabulary. Of more immediate importance will be its contribution to sound medical treatment in earth's colorful sarong, the Tropical Zone.

The Ph.I. will bring order out of chaos in drug standardization, but unfortunately not in spelling. In the United States, the word Pharmacopœia is used in the official seal and articles of incorporation, and Pharmacop*eia* is official for all other purposes. The World Health Organization has made the official Latin spelling Pharmacop*oea*, and its official English translation Pharmacop*oeia*. . . . In case the four official spellings of a single word should provoke grand mal attacks in an editor, it may be helpful to remind him that on page 220 of the 1678 Pharmacopœia Londonensis, the horn of the unicorn is stated to be a powerful remedy for "the falling sickness" (as well as for pestilence, plague, measles, and smallpox.)

F. C. LENDRUM

The Cost of Sickness and the Price of Health, by C.-E. A. WINSLOW, DR. P.H., Consultant in Public Health Administration, W.H.O., Professor Emeritus of Public Health, Yale University. 106 pp. World Health Organization: Monograph Series No. 7 (available also in French), 1951, $1.50; 7s, d., Sw. fr. 6.00.

This study was prepared as a basis for the economic evaluation of preventive medicine which is to take place during the coming Fifth World Health Assembly and which will be followed by a more detailed consideration of the methodology of health protection for local areas.

Professor Winslow has provided separate sections on:

1. The cost of sickness;
2. Methods of reducing the burden of disease, and the economic results attained;
3. Planning a national health programme and its cost;
4. Interrelationships of poverty and disease;
5. Programme of technical assistance.

The presentation is of particular value for two reasons. Discussion in each section is preceded by an historical approach to the specific topic. Probably more valuable to the student of public health economics is the excellent bibliography following each section. The study is so factual that it must be read to be appreciated. The Monograph is a synthesis of figures and estimates culled from the literature and ranges from the economic losses involved in premature deaths to annual losses of countries like India from such specific diseases as malaria. Among the many figures cited are the costs of planning a purely preventive national health service, which he puts at 0.5 per cent of the national income.

The section on the methods of reducing the burden of disease approaches this subject in a rather unique way by first reviewing the achievements in the various fields of public health. The planning of a national health program emphasizes the problem of adequate financing, which is a factor omitted by many who plan without reference to the economic practicability of nationwide application of their proposals. The section on interrelationships of poverty and disease emphasizes the necessity of integrating health programs with concurrent social, educational and economic developments. The last section on the Program of Technical Assistance is an excellent summary of an international development since the last war and probably one which relatively few people are familiar with.

Professor Winslow's term of reference limited his study to providing a basis for consideration of the development of public health administration in particularly underdeveloped areas. It seems unfortunate that the reference could not have been on a broader basis to include as detailed considerations of provision of medical care and the training of all categories of medical personnel instead of being limited to the more conventional scope of public health as held in developed areas. The problem of providing health care in countries of relatively low economic level almost necessitates an over-all rather than a fragmentary approach. However, Professor Winslow has produced a monograph which undoubtedly will have the wide international reference utilization which it deserves.

JOHN B. GRANT

The Infectious Diseases of Domestic Animals, by WILLIAM ARTHUR HAGAN, D.V.M., D.Sc., Professor of Bacteriology and Dean of the Faculty, New York State Veterinary College; and DORSEY WILLIAM BRUNER, D.V.M., Ph.D., Professor of Bacteriology, New York State Veterinary College, Cornell University. Comstock Publishing Company, Inc., Associated with Cornell University Press, Ithaca, New York, 1951, 920 pp.

The second edition retains the logical, lucid presentation with the properly proportioned emphasis which characterized the first edition. It contains 250 more pages than the first edition and these pages are efficiently used to include an accumulation of newer knowledge. As one expected, and as it should be, a large portion of the additional pages (100) has been devoted to virus diseases. It is in this field of infectious diseases that the greatest contribution to our advancement has been made.

Several new chapters have been included in the new edition, and there is no chapter in which some minor or important change or additions have not been made. New tables and larger photographs and additional references are found. In some instances the number of references has been doubled.

A short 10-page section on chemotherapy devoted largely to antibiotics has been added. The scope of the book does not permit more space for this subject. The authors, however, recognized its importance and have included a short paragraph on chemotherapy for each disease.

In a satisfactory manner some changes of classification and nomenclature have been made. The authors have not entirely adhered to the classification in Bergey's "Manual of Determinative Bacteriology." As in the first edition, the second contains reasons for retaining the older terminology in several instances. To some there appears justification for the position taken by the authors in certain instances. The newer classification, however, is referred to in most cases, and the users of the textbook will have it available. In presenting the viruses the text shows no attempt to classify them but instead, as in the first edition, the groups adopted by Topley and Wilson, which are more or less based on tropism, are used.

The table of contents shows the extent of the subject matter treated in the seven parts into which the book is divided: 1. Mechanism of Infection and Resistance; 2. Chemotherapy; 3. Pathogenic Bacteria (and here these are mostly arranged in groups); 4. Bacteria-like Pathogenic Organisms of uncertain classification which include: (a) Spirochaetes, (b) Borrelia, (c) The Bartonella group, (d) The Rickettsiae, (e) The Pleuropneumonia group; 5. Pathogenic Fungi; 6. Pathogenic Protozoa; 7. The Viruses.

In general, in presenting each group of infectious agents there are one or more paragraphs on general statement (which contains a reasonable amount of history including the names of some of the workers associated with the group of organisms); synonyms; morphology and staining reactions; cultural features; resistance; pathogenicity for various domestic and experimental animals and man (here excellent photographs of the pathology are included in a limited and proper proportion). There are paragraphs on diagnosis including bacteriological, serological, hypersensitivity and other tests; immunity covering natural and artificial. These are comprehensive but briefly presented, and in no instance are details of methods or techniques included. Finally, the chapters are concluded with paragraphs on chemotherapy and control measures.

There are, of course, some minor and for the most part insignificant disagreements one can have with the authors. For instance, it might be pointed out that the detail of giving the initials of the reporters in the list of references is omitted.

The Infectious Diseases of Domestic Animals by Hagan and Bruner is highly recommended for students as a text and reference book in microbiology of infectious diseases of animals, and to others interested in various phases of these diseases. It is also a useful and ready reference for those interested and concerned with human infectious diseases, especially those transmitted from animals.

J. Traum

Yellow Fever in Galveston, Republic of Texas, 1839. An account of the great epidemic, by Ashbel Smith, M.D., ex-Surgeon General of the Texian Army, together with a biographical sketch by Chauncey D. Leake, and stories of the men who conquered yellow fever. 135 pp., illustrated, Austin: University of Texas Press, 1951, price $2.50.

The nickname, "Ashbarrel" Smith, conferred by medical students, gives some indication of the salty character of the man, who won eminence as a military surgeon, pioneer practitioner, medical teacher and politician in Texas. In this little volume is republished his interesting contemporary account of a sharp outbreak of yellow fever in Galveston in the autumn of 1839. A graduate in medicine of Yale, Ashbel Smith had gone to Paris for the best available postgraduate training in clinical medicine and pathology. His skill in these branches of medicine is revealed by the careful clinical descriptions of yellow fever cases and the detailed notes on postmortem examinations reported. His observations during this epidemic convinced him that yellow fever is not contagious, but he got no idea of the true epidemiology of the disease.

Most of the account is devoted to clinical observations, to detailed descriptions of gross pathology, and to a discussion of therapy. At first his treatment was simple, but drastic. "I bled and prescribed calomel." The unfortunate results of this regime quickly made him adopt a milder course of treatment which proved more satisfactory.

Dr. Leake has performed a useful service in making this account available and in calling attention in his biographical introduction to the interesting career of Ashbel Smith. The final section of this volume, devoted to notes by various authors on "The Men Who Conquered Yellow Fever," is too sketchy and incomplete to be really satisfactory. The inclusion of this section detracts from the main purpose of the volume.

HUGH H. SMITH

Biochemistry and Physiology of Protozoa, edited by ANDRE LWOFF, head of the Department of Microbial Physiology, Pasteur Institute, Paris. Volume I, pp. 434. New York, Academic Press, Inc., 1951. Price, $8.80.

In contrast to vertebrates and to bacteria, relatively few investigations have been carried out on the metabolism and the nutrition of Protozoa. Such information is scattered widely throughout the zoological, parasitological, microbiological, physiological and biochemical literature. Therefore, the recent book on the biochemistry and physiology of protozoa, edited by A. Lwoff, is to be welcomed because it summarizes and reviews the present status of our knowledge in this field. Also, it undoubtedly will stimulate further much needed research on these problems. As stated by A. Lwoff in his introduction, some biochemists who had been "forced during World War II to cooperate with parasitologists in the study of parasitic protozoa were anxious to return to studies on muscle or yeast. For the future development of protozoan biochemistry, it seems of utmost importance that an atmosphere develop in which more biochemists may feel, without external pressure, that many problems of biochemistry of Protozoa are now ripe for further investigations" and are "quite ready to respond to their love and interest." The authors of each chapter are experts whose original and frequently pioneering investigations are responsible to a large extent for the advances in the particular field reviewed by them. Of particular interest to the parasitologist are the monographs on the metabolism of trypanosomes by T. Von Brand, on the nutrition of parasitic flagellates and of amebae by M. Lwoff and on the biochemistry of plasmodia and the influence of antimalarials by R. W. McKee. These sections of the book reveal certain common patterns in the nutrition and biochemistry of the parasite with their hosts and with other forms of life. On the other hand, the reader is impressed equally by the great number of qualitative and quantitative differences in the metabolic reactions and in the nutritional requirements not only between the mammalian host and the parasite, but also among the various species of parasitic Protozoa. Such and other as yet unexplored differences could explain readily the differential toxicities of chemotherapeutic agents to the host and the parasite, as well as the great variations in drug sensitivity from one parasitic species to another. Continued investigations based on the information contained in this book might reveal a greater number of metabolic reactions which are essential for survival and reproduction of the parasite but which play no role or only a minor one in the host. In this manner more opportunities would become available for inhibiting such reactions by compounds of low mammalian toxicity and for the rational development of chemotherapeutic agents against parasitic diseases.

ERNEST BUEDING

Biological Antagonism. The Theory of Biological Relativity, by GUSTAV J. MARTIN, Sc.D., Research Director, The National Drug Company, Philadelphia, 516 pages, 64 figures, 44 tables, Philadelphia: The Blakiston Company, 1951. Price, $8.50.

Dr. Gustav J. Martin has performed a notable service to biochemists and medicinal chemists in bringing up to date a well organized review of the available information on metabolites and their antagonists. The author has a firm conviction that new medicinal agents can and therefore ought to be conceived rationally from a knowledge of the enzymatic reactions of normal cell metabolites and their selective inhibition by suitable antagonists. That this knowledge should make practical chemotherapy possible rests on two principal premises which the author emphasizes throughout the book:

> "The first concerns the relativity of enzyme inhibitors, the selectivity of antimetabolites based upon structures bearing similarity to natural substrates, cofactors, etc. which are normally involved in vital enzymatic transformation. The second is that of the relative concentration of susceptible enzymes in various tissues and the assumption that any given inhibitor will act primarily against that tissue containing a minimum of the enzyme."

Despite the tremendous volume of papers that have been published on metabolite antagonists in the last few years, Dr. Martin has to a remarkable degree abstracted the current literature. An indication of the magnitude of the recent work is found in comparing the size of Dr. Martin's book with that of the excellent review by Dr. R. O. Roblin, published in Chemical Reviews in 1946. Dr. Martin has indeed set himself a formidable assignment if he intends to maintain his coverage of current literature in future revisions of the volume.

The book begins with a perfunctory outline of the kinetics of enzyme catalyzed reactions and from there plunges directly into an abstracting of the results without any discussion of the procedures on which the various authors' conclusions are based. This knowledge is assumed. The treatment of papers is necessarily superficial and noncritical although Dr. Martin does not hesitate from time to time to express a personal opinion. The organization of the book is excellent, treating the antagonists of the natural metabolites beginning with choline, epinephrine, and histidine, the vitamins treated individually, the amino acids, purines, pyrimidines, steroids, lipids, thyroxine and inorganic ions. The book will be most useful as an index to the recent literature and the reader must be expected to evaluate independently the significance of the reported data.

The subject matter is well organized and the well prepared tables of structural formulas are most helpful. There are numerous typographical errors to be expected in a first edition. Many of Dr. Martin's challenging statements will be disputed by the critical reader. It should be noted that Dr. D. W. Woolley has just written a comparable but shorter book, "A Study of Antimetabolites" published by John Wiley & Sons, Inc.

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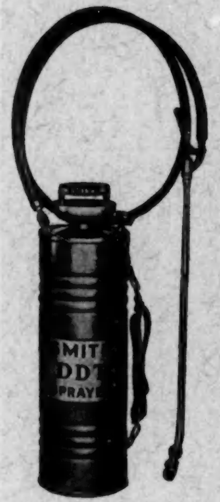

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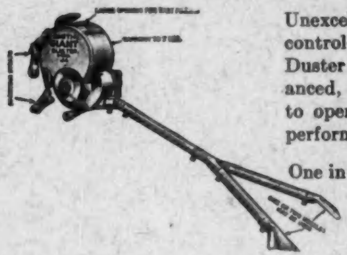

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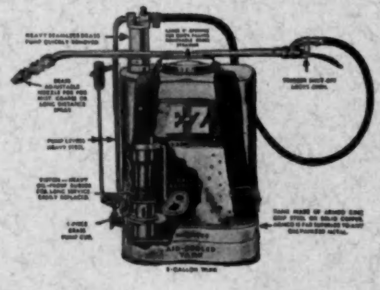

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